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# PATENT COVER SHEET FOR PROVISIONAL APPLICATION

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TITLE OF THE INVENTION (280 characters max)

COMPOUND FUNCTIONALIZATION BY ACTIVITY IN VIVO

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- ☒ Specification (Including Any Claims and Abstract) - 135 pages
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# COMPOUND FUNCTIONALIZATION BY ACTIVITY *IN VIVO*

## FIELD OF THE INVENTION

[0001] The invention relates generally to the *in vivo* testing of the efficacy of a compound or composition, and particularly to the testing and biologically functionalizing of classical small molecules, natural products, genes, peptides and proteins by activity *in vivo.*

## BACKGROUND OF THE INVENTION

[0002] Pharmaceutical companies are interested in evaluating and understanding the function and regulation of newly discovered genes and gene products (proteins), especially newly discovered genes and proteins, which could help in the understanding of the mechanisms linked to diseases or compounds action. In addition, the genes and gene products can become potential drugs or biomarkers. Grenet O, *Pharmacogenomics J.* 1(1):11-2 (2001).

[0003] However, a gene sequence alone does not provide information about the actual function of the protein in the cell or organism physiology. In addition, while the genome has a relatively well defined number of genes, there is no known limit to the possible number of protein variants. The potential number of proteins encoded by these genes is estimated to be from two to at least one-hundred times higher than the number of genes, since it has recently been found that proteins can also be produced by splicing at the protein level not just at the RNA level.

[0004] The current process of drug discovery proceeds from single target to single drug product. The current process is a long process, frequently with late attrition for lack of efficacy, wrong design or false indication.

[0005] Thus, there is a need in the art for a more efficient method for discovering and identifying drug candidates, gene targets and biomarkers.

## SUMMARY OF THE INVENTION

[0006] The invention provides a discovery process for biologically functionalizing peptides, proteins, genes, small molecules and natural products using organism-wide gene expression profiling. The discovery process of the invention proceeds from single lead or drug to multiple targets and indications (as indicated by an impact on any target in the cascade chain of a pathway), and multiple drug products, thus providing rapid guidance to a correct human proof-of-concept.

[0007] The discovery process of the invention begins with administration of test substances to animals, followed by screening of the resulting gene expression in many organs obtained from the test animal. The invention can be used to biologically functionalize the entire genome of any organism where micoarray chips are available. The invention is not restricted to the type of compound to be functionalized. Small molecules, proteins, natural products, cDNA (for functionalizing any gene of interest), *etc.* are all susceptible to the strategy of the invention.

[0008] Since the discovery process of the invention is based on a non-preconceived hypothesis and whole organism multi-organ analysis, polypeptides can be selected for testing in the absence of any biological selection criteria other than peptide sequence. The resulting organism-wide pattern of the gene expression changes in the transcriptome provides an overview of the activities at the molecular and organism-wide levels. Accordingly, the unbiased approach of the invention regarding the administration of a compound can provide information about the physiological relationships throughout the entire body that are caused by the compound's administration.

[0009] The discovery process of the invention then integrates *in vivo* profiling with internal and external genomic databases to elucidate the function of unknown proteins, typically within few months. The unbiased approach of the invention in regards to the administration of a compound advantageously provides genomic signatures from multiple organs. The resulting data can be analyzed either by using tools that are known to those of skill in the art or by using tools that compare the compound signatures produced by the administration of the compound among the different organs. This multi-organ analysis is in contrast to standard approaches, which, since they do not use an unbiased approach of compound administration, do not result in a multi-organ identification of the function of the compound. Instead the standard approaches provide analysis on a case-by-case basis, which can make cross-experimental comparisons difficult. In contrast to the standard approaches, the identification of the function of the compound using the method of the invention allows for the identification of the function of the compound in many metabolic and regulatory pathways. In addition, the identification of the function of the compound using the method of the invention advantageously results in an understanding of the stability of the active compound in the body, a property of the administered compound which would otherwise not be predictable *a priori* using standard approaches.

[0010] The identification of the function of the compound using the method of the invention can be multi-step, as one step or part of the identification leads to another step or part of the identification, to provide a more complete understanding of the administered compound's activity *in vivo*. For example, the identification of compound function in one organ (such as the spleen) can lead to an understanding of the compound function in other organs. By contrast, the standard approaches, which rely on immediate access to tests on a limited number of organs, depend on anecdotal evidence from other experiments to further steps in the identification of compound function.

[0011] The invention is suitable for several stages of drug discovery, identifying both drug targets and biomarkers. The discovery process of the invention advantageously delivers an increased number of validated drug candidates and identified drug targets and biomarkers along with a savings in time, resources and animals. The discovery process of the invention advantageously integrates into one process standard exploratory tools with new genomic approaches. The discovery process of the invention can also be used for the reprofiling of safe compounds stopped after the initial stages of drug approval (*e.g.*, Phase I) for re-indication. The invention can be used for adjusting the best fit for combination therapies, by optimally matching of gene expression signatures between two compounds, canceling of side effects and the potentiation of efficacy. The discovery process of the invention can be used for the profiling of the more advanced development portfolio to guide the later stages of drug approval process (*e.g.*, Phase II and Phase III).

[0012] In several embodiments, the process can be used to analyze tissues or body fluids (such as coronary heart disease, breast cancer and another indication; each compared to healthy controls). Plasma proteins that are differentially expressed between normal subjects and coronary artery diseased patients, with known or unknown function, are analyzed for potential target identification/validation, and biomarker identification.

[0013] In one embodiment, the discovery process of the invention begins with an *in vivo* screening of proteins, peptides and reference compounds in mice. Based on the results of the mice screening, an *in vivo* verification of selected proteins, peptides or reference compounds is then conducted in non-human primates or animal models of human pathology or disease. The comparison of the resultant information to a profile of reference drugs, with well characterized pharmacological activity, facilitates biological interpretation of the profiles of unknown compounds. In a particular embodiment, the selection rate for the proteins, peptides or reference compounds is ~20%.

[0014] In one embodiment the discovery process of the invention combines in one process: (a) pre-screening in mice; (b) verification of selected proteins/peptides/reference compounds in monkeys; (c) large number of the analyzed tissues (up to 25 in mice, up to 120 in monkeys); (d) homogeneity of the tissue sample (e) high quality mRNA; (f) a genome-wide approach with hybridization chips; (g) powerful bioinformation tools for clustering and statistics; (h) the possibility of cross-assay meta-analysis; and (i) localization at the cellular level of the affected genes or pathways by *in situ* hybridization.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0015] FIG. 1 is a set of polypeptide sequences and putative polypeptide sequence correlations for GPA019, GPA020, GPA021, GPA022 and GPA023.

[0016] FIG. 2 is a chart showing the activity of GPA018 in the kidney, listing differentially expressed genes with relation to TGFβ signaling.

[0017] FIG. 3 is a bar graph showing the increase in endometrial thickness following the administration of estrogenic compounds to ovariectomized monkeys.

[0018] FIG. 4 is a bar graph showing the increase in endometrial epithelial height following the administration of estrogenic compounds to ovariectomized monkeys.

[0019] FIG. 5 is a bar graph showing the increase in endometrial epithelial height following the administration of estrogenic compounds to ovariectomized rats. Doses are in mg/kg p.o.

[0020] FIG. 6 is a bar graph showing the increase in endometrial epithelial height following the administration of estrogenic compounds to ovariectomized rats. Doses are in mg/kg p.o.

[0021] FIG. 7 is a line graph showing the levels of serum C-telopeptide following the administration of estrogenic compounds to ovariectomized monkeys.

[0022] FIG. 8 is a line graph showing the levels of serum alkaline phosphatase following the administration of estrogenic compounds to ovariectomized monkeys.

## DETAILED DESCRIPTION OF THE INVENTION

[0023] *Introduction and overview.* The classical discovery process in the pharmaceutical industry is based on targets (enzymes, receptors, cellular assays, animal and disease models, *etc.*). Chemicals or biological products are tested, in a high-throughput mode, on a battery of pre-selected different targets. The weakness of the classical approach are the "artificially

disconnected" *in vitro* target models compared to the tightly interconnected and interdependent relationship of the different targets in a whole organism and the fact that biological activity on all non selected targets is missed.

[0024] By contrast, the invention is a "non pre-conceived hypothesis" discovery process to rapidly identify and analyze the biological activity of new products in the whole organism, multi-organs and whole transcriptome. All physiological interactions between the different organs or tissues are present and any cellular pathway or any potential targets could potentially be analyzed in a non artificial system.

[0025] The drug discovery process of the invention advantageously increases the capabilities in the field of proteomics and functionalization. Proteomics involves the systematic separation, identification and characterization of the proteins present in a sample of tissue, or in a biological fluid, at a given time. All biological processes, including diseases and responses to drugs, induce changes in proteins, and the global protein profile (the "proteome") varies during the development of an organism, maturation of cell types or tissues, and progression or treatment of disease. Each cell type may express different patterns of proteins at different times. Each protein in turn may be modified chemically in an equally diverse number of ways to serve different cellular functions. As proteins derived from the same gene can be largely identical, and might differ only in small but functionally relevant details, protein identification tools not only identify a large number of proteins but also differentiate between close relatives.

[0026] The classical proteomics approach combines high resolution two-dimensional gel electrophoresis (2-DGE) with imaging software to quantitatively and qualitatively screen for proteins that differ in abundance, molecular weight (Mr) or charge between the gels. These protein differences can then be identified with high speed and sensitivity by using a combination of "state-of-the-art" mass spectrometry (MS) approaches and robotics, alongside sensitive bioinformatics search tools.

[0027] RNA transcripts represent the intermediate form between the DNA and the proteins that are among the most active molecules involved in the cellular functions. The total content of RNA is called the "transcriptome". The high-density DNA chip technology gives potential access to the analysis of all the transcripts produced by a cell population or tissue at any determined time point. Genome-scale RNA expression analysis can thus provide new insights into the cellular events induced upon administration of an animal with peptides or other chemicals. This provides a broad view of the metabolic, signaling, regulatory or other

biochemical pathways in the animal being tested. The analysis of the induced perturbations in cellular transcription gives a detailed molecular description of the activity of the administered compound.

[0028] An analysis of a transcriptome has become an approachable reality with the implementation of high throughput RNA quantification system. The high-density microarrays allow collecting thousands of information points of a transcriptome at once, reaching the order of magnitude of the probable number of genes expressed and producing a broad and detailed view of the cellular events.

[0029] As the changes of the different functions inside a cell are tightly interconnected, the changes in different organs inside an organism are linked. Applying gene profiling to different organs submitted to the same treatment gives a complete overview of the effects and modifications of the physiological status. The identification of common changes in organs with originally very different transcriptomes facilitates the elimination of the experimental noise. The presence or absence of identical signals can indicate if the treatment has a pleiotropic effect or is affecting a target organ. If a compound is targeting a primary organ, the other organs will reflect the functional modifications of the first organ impacted. This type of information can be collected in correlation to the pharmacological effect or to the potential toxic effects. The organism-wide pattern of expression changes can also provide useful information on the pharmacodynamic of the compound, precisely delineating the range of organs affected.

[0030] The accumulation of information in different organs not only helps to elucidate the precise mode of action but also provides a complete reconstruction of the compound-induced modifications at the organism scale.

[0031] *Administration of compounds*. Administration of the protein or other drug compound triggers multiple cascades of intracellular signaling events, involving complex networks (pathways) and relying on protein modifications such as phosphorylation, glycosylation, *etc*. These events eventually lead to modifications of gene expression levels. Administration of an active compound therefore leads to multiple and interdependent changes in the composition of the transcriptome.

[0032] In one embodiment, the test animal is a vertebrate. In a particular embodiment, the vertebrate is a mammal. In a more particular embodiment, the mammal is a primate, such as a cynomolgus monkey or a human. As used herein, the administration of an agent or drug to a subject or patient includes self-administration and the administration by another.

[0033] In more particular embodiments, natural or synthetic substances of biological or non-biological sources, *e.g.* amino acids, peptides, proteins, nucleotides, cDNAs, chemicals, can be administered to animals, *e.g.* mice (*Mus musculus*), rat (*Rattus norvegicus*), monkey (*Macaca fascicularis*), by methods known in the art, *e.g.* by injection, inhalation, or oral administration. Administration of those substances can be adjusted in terms of time of exposure and dosage, and combinations thereof. The "treatment group" of animals should receive a substance or a combination of substances in a vehicle compound suitable for administration of the substance or the combination of substances, while the "control" (or "baseline") group should receive the vehicle compound only. During the treatment period biological specimen such as tissue pieces (*e.g.* obtained by biopsy), or body fluids, such as blood, urine, or saliva, can be sampled. At the end of the treatment time all animals of all groups can be sacrificed and biological specimen such as whole organs or pieces thereof can be sampled. All sampled specimen can be stored as known in the art for further analysis that include, but are not limited to, RT-PCR, Northern blotting, in-situ hybridization, gene expression profiling with microarrays.

[0034] As used herein, "direct administration" is the injecting, oral gavage, feeding or other administration of a compound, such as a protein, into animals. After some time, *i.e.* hours, days or weeks, organs and tissues are collected from the animals and the gene-expression profiles determined. This procedure is commonly used in pharmacotoxigenomics, pharmacogenomics and the like.

[0035] In one embodiment, the invention begins with differentially expressed proteins in plasma between normal subjects and coronary artery diseased patients with regard to the identification and validation of potential targets and the identification of biomarkers.

[0036] The drug discovery process of the invention is particularly amenable to the analysis of the smaller proteins of a proteome (ranging from 0.5 to 20 kDa) escaping the classical detection methods. Small molecular weight proteins can be readily synthesized by commercial methods (*e.g.*, Microprot™ method, GeneProt, Geneva, Switzerland). Chemically-synthesized proteins can be rapidly produced and do not contain biological contaminants.

[0037] For mice, a minimal amount of the compound to be functionalized (only *ca.* 5 mg) is used.

[0038] As used herein, "indirect administration" is the injecting of a gene that codes for that protein (as a cDNA plasmid) and then doing the gene expression profiling. In one

embodiment, the technology is the use of 'naked' DNA (a cDNA expression plasmid) injected into mice (or other animals). This technique is widely published for either DNA immunization (Kim J-M *et al., Gene Ther.* 10(15): 1216-24 (August 2003)) and delivery of genes for therapeutic purposes (Aliño SF *et al., Gene Ther.* 10(19):1672-9 (September 2003)). Among a number of techniques for gene transfer *in vivo*, intravenous injection or the direct injection of plasmid DNA into muscle are simple, inexpensive, and safe. Kim J-M *et al., Gene Ther.* 10(15): 1216-24 (August 2003). The important efficacy of nonviral genomic DNA opens a new avenue in the safety applications of human gene therapy. Aliño SF *et al., Gene Ther.* 10(19):1672-9 (September 2003).

[0039] Administration of naked DNA can be by methods known to those of skill in the art, see, U.S. Pat. Nos. 6,165,754; 6,309,370; 6,566,342; 6,620,617 and 6,651,655, and references cited therein.

[0040] *Gene expression profiles.* After a period of time (*e.g.*, two weeks) of protein administration, the treated animals are necropsied. Selected tissues (*e.g.*, 25 tissues for mice/ 120 tissues for monkeys) are dissected and rapidly snap-frozen for genomics analysis. Organ samples (*e.g.*, fifty organs samples for monkeys) can be isolated for histopathological examinations and for gene expression localizations, such as by *in situ* hybridization. Initial studies have shown that for mice, 3-10 tissues out of twenty-five sampled tissues are generally sufficient to characterize a compound by gene expression and hybridization; for monkeys, twenty tissues out of 120 sampled tissues are generally sufficient.

[0041] In more particular embodiments, the methods of detecting the level of expression of mRNA are well-known in the art and include, but are not limited to, reverse transcription PCR, real time quantitative PCR, Northern blotting and other hybridization methods. A particularly useful method for detecting the level of mRNA transcripts obtained from a plurality of genes involves hybridization of labeled mRNA to an ordered array of oligonucleotides. Such a method allows the level of transcription of a plurality of these genes to be determined simultaneously to generate gene expression profiles or patterns.

[0042] As used herein, a gene expression profile is diagnostic when the increased or decreased gene expression is an increase or decrease (*e.g.*, at least a 1.2-fold difference) over the baseline gene expression following administration of a compound. As used herein, a gene expression pattern is "higher than normal" when the gene expression (*e.g.*, in a sample from a treated subject) shows a 1.2-fold difference (*i.e.*, higher) in the level of expression compared to the baseline samples. A gene expression pattern is "lower than normal" when the gene

expression (*e.g.*, in a sample from a treated subject) shows a 1.2-fold difference (*i.e.*, lower) in the level of expression compared to the baseline samples. In other embodiments, a 1.5-fold change may be used as the criteria.

[0043] Techniques for the detection of gene expression of the genes described by this invention include, but are not limited to northern blots, RT-PCT, real time PCR, primer extension, RNase protection, RNA expression profiling and related techniques. Techniques for the detection of gene expression by detection of the protein products encoded by the genes described by this invention include, but are not limited to, antibodies recognizing the protein products, western blots, immunofluorescence, immunoprecipitation, ELISAs and related techniques. These techniques are well known to those of skill in the art. Sambrook J *et al.*, *Molecular Cloning: A Laboratory Manual, Third Edition* (Cold Spring Harbor Press, Cold Spring Harbor, 2000). In one embodiment, the technique for detecting gene expression includes the use of a gene chip. The construction and use of gene chips are well known in the art. See, U.S. Pat Nos. 5,202,231; 5,445,934; 5,525,464; 5,695,940; 5,744,305; 5,795,716 and 5,800,992. See also, Johnston, M. *Curr Biol* 8:R171-174 (1998); Iyer VR *et al., Science* 283:83-87 (1999) and Elias P, "New human genome 'chip' is a revolution in the offing" *Los Angeles Daily News* (October 3, 2003).

[0044] Gene expression profiles can be generated using *e.g.* the Affymetrix microarray technology. Briefly, total or, preferably, polyA+-RNA from a biological sample is extracted using standard procedures known in the art, *e.g.* the RNeasy® kit (Qiagen, MD, USA). In a following step, double stranded cDNA is prepared in a process termed "reverse transcription (RT)" which is known in the art, using e.g. the "SuperScript Double-Stranded cDNA Synthesis Kit" (Invitrogen, CA, USA). In a subsequent step, termed "in-vitro transcription", double stranded cDNA obtained in a previous step is labeled with a fluorochrome by methods known in the art, using e.g. the ENZO Labeling Kit (ENZO, NY, USA). Labeled RNA is hybridized to oligonucleotide microarrays. These are known in the art and consist of a surface to which probes that correspond in sequence to gene products (*e.g.* mRNAs, polypeptides, fragments thereof etc.) can be specifically hybridized or bound to a known position. Processing of the microarrays, including e.g. washing, staining, scanning, is performed according to the manufacturer's instructions. Hybridization intensity data detected by the scanner are automatically acquired and processed by analytical software components, *e.g.* the GENECHIP® software (Affymetrix, CA, USA). Raw data is normalized to expression levels using a target intensity of 200.

[0045] Two elements of value in expression profiling are the quality and homogeneity of the tissue samples and the mRNA quality. For this purpose, the location of tissues to be sampled and each sample can be carefully dissected from the other surrounding tissues using a binocular microscope.

[0046] The samples are then transferred to a molecular biology laboratory for RNA extraction. The protocol for RNA extraction can be partially automated thus increasing the reproducibility and speed of this step. The extracted RNA can be stored for long periods of time in a frozen state and kept as an archive.

[0047] An aliquot of the extracted RNA is reverse transcribed to obtain a cDNA. In a second step, cDNA is transcribed in the presence of a fluorescent label to obtain cRNA. The composition of the cRNA obtained is identical to the original composition of the RNA in the samples, but each molecule now carries a fluorescent marker. The labeled mixture of cRNA is used for the hybridization process, *e.g.* using GeneChip® assays (Affymetrix, Santa Clara, California USA). The raw data (obtained after laser-scanning of the chip) are processed by a specific algorithm condensing for each gene all available information in a unique value. This value called average difference represents the level of expression of the gene.

[0048] The information can be further refined by the use of complementary techniques. *In situ* hybridization, for example, can indicate precisely which cell type inside an organ is specifically expressing a given gene. This technique based on the detection of RNA is independent of the availability of an antibody. Quantitative PCR may also be used to confirm expression levels of particular genes of interest.

[0049] *Analysis*. Mathematical and statistical processing of the data (clustering) help to reduce the complexity and the size of the data sets. Different types of clustering can be used to separate the different genes according to their behavioral similarity across the different conditions and to establish links between genes that may be related to the same biological phenomenon. Data processing also includes statistical tests to separate significant variations from experimental noise. However, the stringency of the various filtering steps must be modulated to integrate the biological nature of the data.

[0050] The list of different affected genes is then compared to the information collected in the scientific literature. The synthesis of the available knowledge related to the different genes, points to one or several signaling, metabolic or other biochemical pathways or to known modifications. Once a coherent picture has been reconstructed, the profiles may be

associated with potential indications. The discrimination between the different hypotheses follows a process closely related to differential diagnosis.

[0051] During the analysis, a constant comparison between the expression data and the current knowledge on cell signaling and regulation is established. Such a permanent bridge provides an efficient way to refine the existing models, particularly in the field of intra- and inter-cellular signaling. The interdependence of gene expression changes is assessed in different organs and under different stimuli. New players in the pathways can be identified and the link between the already described players can be refined. Even if only a part of the cellular regulation depends on the RNA expression changes, the accumulation of expression data can help to build new and more accurate model of the cell functions. The information collected could help to identify the critical elements of the pathways to be used as target or biomarker.

[0052] Some of the expression profiles can be easily matched with existing information harvested from the general scientific knowledge. Linking this information with a potential indication or a potential side effect is then straightforward. Some combinations of expression changes are more difficult to translate into pharmacological information. In such cases, matching of the RNA expression profile of an unknown compound to the profile of a reference drug or disease may facilitate the interpretation. It may then not be necessary to reconstruct the entire cellular modifications to find a potential indication. The reference drugs and disease profiling will also help to build the critical mass of information into the database.

[0053] In more particular statistical analysis embodiments, microarray datasets can be analyzed by the use of analytical software components, such as GeneSpring® (Silicon Genetics, CA, USA). Microarray datasets consist in part of probe set identifiers that refer to an oligonucleotide sequence that is bound to the glass slide and to which a labeled cDNA (see above) with complementary sequence binds if it is present in the tissue or body fluid sample. The scanned intensity of the signal that is detected and converted into numeric values by a software, for example MAS5 (Affymetrix, CA, USA), is an indirect measure for the amount, or expression level of the cDNA present in the biological samples under investigation. The entity of gene expression levels as indicated by signal intensity values for all probe sets in a microarray dataset of a biological sample can be referred to as expression profile of that sample. In each microarray dataset, signal intensity values, cDNA or gene annotations, as well as quality parameters that can be created by software, for example MAS5 (Affymetrix, CA, USA), are informational results associated with the probe set ID.

[0054] In the cDNA microarray system, expressions of genes from the experimental cells of interest are measured relative to the expressions of the same genes in a fixed reference or control cell type. To identify statistically relevant effects of a substance on the expression profile of samples or tissue or body fluid under investigation, probe sets can be filtered based on the associated values given by the software used to create the values, for example MAS5 (Affymetrix, CA, USA). Filters can be based on quality parameters, expression level, changes of expression levels in the samples from treated versus control specimen, as well as significance. The resulting list of probe sets refers to such genes that experience a significant change in their expression level as a direct or indirect result of the treatment of the biological samples they are derived from.

[0055] The interpretation of such gene lists with regards to effects of a substance on biological systems and pathways is subject to the investigators knowledge and experience. Application of analytical software components such as GeneSifter® (VizXLabs, Seattle,WA, USA) assists in the interpretation of such gene lists.

[0056] Moreover, we have developed software for a multiorgan analysis of the data generated by the method of the invention, which compares the compound signatures produced by the administration of the compound among the different organs.

[0057] The following EXAMPLE is presented in order to more fully illustrate the preferred embodiments of the invention. This EXAMPLE should in no way be construed as limiting the scope of the invention, as defined by the appended claims.

EXAMPLE I

INTEGRATED INVESTIGATIVE PHARMACOLOGY THROUGH *IN VIVO* GENE EXPRESSION PROFILING IN MICE

[0058] In this EXAMPLE, 100 unknown chemically synthesized peptides are functionalized using the discovery method of the invention. Most of these peptides are present in human plasma.

[0059] As a control, twenty reference drugs are concurrently investigated with the discovery method of the invention. For this screening, one control and four treated groups of six males are treated for two weeks by daily administration by the subcutaneous route of the proteins. The reference drugs can be active for treating conditions in the areas of glaucoma, neuroprotection, neovascularisation, antiangiogenesis, acne, asthma and allergy,

cardiovascular diseases, neurological disorder, pain, diabetes, hypercholesterolemia, osteoporosis and oncology.

[0060] The expectations from those selected active peptides are that (a) several potential therapeutic drugs could be identified; (b) target peptides for therapeutic antibodies could be identified; (c) new targets for research, deduced from the reconstructed biochemical pathways, could be identified; and (d) biomarkers, to be used to develop diagnostic tests could be identified. Bioinformatic investigations of gene expression in mice suggest therapeutic indications and insight through the analysis of molecular pathways and functions, which allow prioritizing for the verification of selected proteins/peptides in monkeys. The protein/peptide selection rate is approximately 20%. The selection/prioritization criteria are the type of activity, the therapeutic interest and the suspected toxicity.

[0061] In an initial analysis, following administration of a peptide (FGF23CTP, *see below,* EXAMPLE III) to mice, transcript level changes were observed in several organs which indicate that the compound is active and impacts on pathways involved in cell differentiation.

EXAMPLE II

A 7-DAY PHARMACOLOGY AND TOXICITY STUDY BY SUBCUTANEOUS ROUTE IN MICE; MICROARRAY GENE EXPRESSION ANALYSIS

[0062] *Introduction and summary.* Five peptides with unidentified function were tested in mice to obtain biochemical and pharmacogenomic data that would allow a specification of their activity. Outbred CD-1 mice were treated with peptides GPA018, GPA019, GPA020, GPA022, and GPA023 for seven days, observed for clinical signs of treatment effects (mortality, clinical signs, body weight, food consumption, hematology, clinical biochemistry) and, after sacrifice, a selected set of tissues were used for gene expression profiling. A snap freezing sampling of the tissues was performed at necropsy at the end of the treatment period. These tissues were used for mRNA expression profiling and for histopathological analysis (formalin fixation). In addition, parameters investigated in a standard exploratory study were recorded. None of the peptides had any influence on clinical or pharmacogenomic parameters. Gene expression profiling revealed no significant changes between control and treated animals. It was concluded that the peptides were inactive and decided that no further investigations on these peptides would follow.

[0063] *Treatment*. Peptides GPA018, GPA019, GPA020, GPA022, and GPA023 (GeneProt, Geneva, Switzerland; see, FIG. 1) were administered subcutaneously to CD-1 mice for seven days at a dose of 300 mcg/day. The choice of CD-1 mice (an outbred strain from Charles River Laboratories, l'Arberesle, France) was made to increase the mouse organ weight and, hence, yield of RNA for microarray analysis. Four animals per treatment arm and gender were used. At the beginning of the treatment period, the animals were 12 to 14 weeks old. Body weight averaged 42.2g (38 to 45.6 g). Animals were kept under standard conditions for animal welfare.

[0064] Dosage forms were prepared once before the beginning of the treatment period. Each test item was dissolved in the vehicle (PBS) in order to achieve the required concentration. The dosage forms obtained were divided into aliquots and stored at -20°C pending use. Two aliquots were prepared for each group and day. The aliquots for treatment were delivered twice on each day of treatment to the animal room.

TABLE 1
Animal Allocation and Test Item Dosages

| Treatment | Number of animals | Dose-level (µg/kg/day) | Concentration (µg/mL) |
|---|---|---|---|
| Control | 4 male and 4 female | 0 | 0 |
| GPA018 | 4 male and 4 female | 150 x 2 | 15 |
| GPA019 | 4 male and 4 female | 150 x 2 | 15 |
| GPA020 | 4 male and 4 female | 150 x 2 | 15 |
| GPA022 | 4 male and 4 female | 150 x 2 | 15 |
| GPA023 | 4 male and 4 female | 150 x 2 | 15 |

[0065] *In vivo examinations*. Animals were examined at least twice daily for mortality, food consumption and clinical observations. Body weight was recorded once per week.

[0066] Blood samples were collected from each animal. The serum samples were deep frozen (approximately -80°C) until analyses for hormone determination.

[0067] For tissue sampling, animals were asphyxiated by carbon dioxide approximately 12 to 16 hours after the last injection. A gross macroscopic post-mortem examination was performed in order to specify the possible reduced size of main organs (with particular attention to lymphoid tissues). No treatment-related morphological changes were noticed.

[0068] Snap freezing of many organs was performed at necropsy at the end of the treatment period. Within 15 to 20 after the sacrifice, all sampling for snap freezing was performed.

[0069] Twenty-eight tissues were sampled, including brain, duodenum (caecum), liver, kidney, muscle and spleen (blood). For this EXAMPLE, male and female animals were used in the kidney and in the liver assays. All other tissue samples were derived from males only.

[0070] Samples for histopathology were fixed in phosphate-buffered 10% formalin. Bone demineralization was performed with 10% formic acid.

[0071] Samples for gene expression profiling were quick-frozen in liquid nitrogen immediately after excision, stored on dry ice and subsequently in a deep-freezer at approximately -80°C until further use.

[0072] *RNA extraction and purification*. Briefly, total RNA was obtained by acid guanidinium thiocyanate-phenol-chloroform extraction (Trizol®, Invitrogen Life Technologies, Carlsbad, Calif. USA) from each frozen tissue section and the total RNA was then purified on an affinity resin (Rneasy®, Qiagen) according to the manufacturer's instructions, and quantified. Total RNA was quantified by the absorbance at $\lambda = 260$ nm ($A_{260nm}$) and the purity was estimated by the ratio $A_{260nm}/A_{280nm}$. Integrity of the RNA molecules was confirmed by non-denaturing agarose gel electrophoresis. RNA was stored at approximately -80°C until analysis. One part of each individual RNA sample was kept for the analysis of critical genes by means of Real-Time PCR.

[0073] *GeneChip® assays*. All GeneChip® assays were conducted as recommended by the manufacturer of the GeneChip system (Affymetrix, *Expression Analysis Technical Manual*, (Affymetrix, Santa Clara, Calif. USA, 2003). Genome MG-U74Av2 expression probe array set (Affymetrix, Inc., San Diego, Calif. USA) were used.

[0074] Double stranded cDNA was synthesized with a starting amount of approximately 5 μg full-length total RNA using the Superscript Choice System (Invitrogen Life Technologies) in the presence of a T7-(dT)24 DNA oligonucleotide primer. Following synthesis, the cDNA was purified by phenol/chloroform/isoamylalcohol extraction and ethanol precipitation. The purified cDNA was then transcribed in vitro using the BioArray® High Yield RNA Transcript Labeling Kit (ENZO, Farmingdale, New York, USA) in the presence of biotinylated ribonucleotides form biotin labeled cRNA. The labeled cRNA was then purified on an affinity resin (Rneasy®, Qiagen), quantified and fragmented. An amount of approximately 10 μg labeled cRNA was hybridized for approximately 16 hours at 45°C to an expression probe array. The array was then washed and stained twice with streptavidin-phycoerythrin (Molecular Probes, Eugene, Oregon, USA) using the GeneChip® Fluidics Workstation 400 (Affymetrix).

[0075] The array was then scanned twice using a confocal laser scanner (GeneArray® Scanner, Agilent, Palo Alto, Calif. USA) resulting in one scanned image. This resulting ".dat-file" was processed using the MAS4 program (Affymetrix) into a ".cel-file". The ".cel file" was captured and loaded into the Affymetrix GeneChip Laboratory Information Management System (LIMS). The LIMS database is connected to a UNIX Sun Solaris server through a network filing system that allows for the average intensities for all probes cells (CEL file) to be downloaded into an Oracle database. Raw data was converted to expression levels using a "target intensity" of 150. The data were checked for quality and loaded into the GeneSpring® software 5.0.3 (Silicon Genetics, Redwood City, Calif. USA) for analysis.

[0076] As a quality control, hybridizations were performed using GAPDH or β-actin probes.

[0077] *Data analysis*. RNA samples were studied by using the human Affymetrix MG-U74Av2 GeneChip®. On such chip platform, probe-sets for individual genes contain 20 oligonucleotide pairs, each composed of a "perfect match" 25-mer and a "mismatch" 25-mer differing from the "perfect" match oligonucleotide at a single base. After probe labeling, hybridization, and laser scanning, the expression level is estimated by averaging the differences in signal intensity measured by oligonucleotide pairs of a given probe (AvgDiff value). The image acquisition and numerical translation software used for this study was the Affymetrix Microarray Suite version 4 (MAS5). The numerical values were stored and transferred for the analysis into the Silicon Genetics GeneSpring® 5.0.3 software toolkit.

[0078] Various filtering and clustering tools in these programs were used to explore the datasets and identify transcript level changes that inform on altered cellular and tissue functions and that can be used to establish working hypotheses on the modes of action of the compound. All datasets were normalized to the median. To identify pleiotropic or unique effects, scripts provided by Silicon Genetics, were applied. Pleiotropic effects were also investigated manually by analyzing genes with an expression level of more than 60 in at least three of eight conditions (*i.e.* organs) and hereafter applying statistical analysis (parametric, assume variances not equal, $p<0.1$) to identify genes with significant changes in their expression between control and treated animals.

[0079] To identify significant changes in gene expression in individual organs, an expression restriction filter was applied (ADV >60 in 50% of the samples), and a statistical filter as described above was added.

[0080] In some instances, a fold change filter script (provided by Silicon Genetics) was used after the expression restriction to find genes with a change in gene expression above a certain level, and then a statistical filter applied.

[0081] The decision to consider a specific gene relevant is based on a conjunction of numerical changes identified by exploratory filtering and statistical algorithms as described above and the relationship to other modulated genes that point to a common biological theme. The weight of that relationship is assessed by the analyst through a review of the relevant scientific literature.

[0082] *Gene expression profiling*. Despite a multitude of statistical approaches, no significant changes in the gene expression patterns of the treatment groups were observed. In every analysis the number of changed genes was always smaller than the number of genes that would have been found by chance. The range of fold change was usually very small, seldom exceeding 2-fold.

[0083] Interestingly, the strongest changes were gender-specific. Comparison of control males and control females, as well as treated males versus treated females, showed about 3 times as many genes changed than did control males or females versus treated males or females. In addition, the genelists of treatment specific effects in females and males showed little overlap.

[0084] In the GPA018 treated kidneys of males and females combined (eight samples per group), some possible influence on genes affected by or affecting TGFβ signaling were observed (FIG. 2 and TABLE 2). GPA018 shows some sequence similarity to the N-terminal region of mLTBP-2 (murine latent transforming growth factor binding protein-2). LTBP proteins aid the LAP (latency associated protein)-TGFβ complex to become secreted and binds it to the ECM (extracellular matrix)-structural protein fibrillin (Annes JP *et al., J. Cell Sci.* 116(Pt2):217-24 (2003); Chen S *et al., Nucl. Acids Res.* 31(4):1302-10 (2003); Vehivilainen P *et al., J. Biol. Chem.* 278(27):24705-13 (2003)). However, the extent of the changes after treatment was very small, usually below 1.5-fold, and similar patterns were not observed when female or male groups were investigated separately.

TABLE 2
GPA018 in Kidney

| Expressed Gene | Comments and Literature |
|---|---|
| αII-spectrin | Cleaved during TGFβ induced apoptosis (Brown TL *et al., J. Biol. Chem.* 274(33):23256-23262 (1999)) |
| β-spectrin | Disruption of β-spectrin ELF leads to disruption of TGFβ signaling in mice (Tang Y *et al., Science* 299(5606):574-7 (2003)) |
| β5-Integrin | TGFβ induces integrin (Ludbrook SB *et al., Biochem. J.* 369(Pt2):311-8 (2003)) |
| Integrin α(v)β3 | Gene is a receptor for the LAP of TGFβ1 and 3 (Ludbrook SB *et al., Biochem. J.* 369(Pt2):311-8 (2003)) |
| TGFβ type I-receptor (ALK-5) | Overexpression downregulates integrin β5 and LTBP1 expression (Ota T *et al., J. Cell Physiol.* 193(3):299-318 (2002)) |
| LEF | TGFβ induces expression of LEF1 target genes; crosstalk between wnt and TGFβ pathway (Hu MC *et al., Development* 130(12):2753-2766 (2002); Letamendia A *et al.,* J. *Bone Joint Surg. Am.* 83-A Suppl 1(Pt1):S31-9 (2001)) |
| Fas Death Domain-Associated Protein (DAXX) | TGFβ–induced apoptosis is mediated by DAXX that facilitates JNK activation (Perlman R *et al., Nature Cell Biology* 3(8):708-14 (2001)) |
| Forkheadbox D2 | Identification of FOXC1 as a TGFβ1 responsive gene and its involvement in negative regulation of cell growth (Zhou Y *et al., Genomics* 80(5):465-72 (2002)) |
| Biglycan | Smad4-dependent regulation of biglycan gene expression by TGFbeta in pancreatic tumor cells (Chen WB *et al., J. Biol. Chem.* 277(39):36118-28 (2002)) |
| Amyloid β precursor protein | TGFβ1 potentates amyloid-β generation in astrocytes and in transgenic mice; induces overexpression of APP (Lesne S *et al., J. Biol. Chem.*278(20):18408-18 (2003)) |
| Transgelin (SM22α) | TGFβ type I-receptor (ALK-5) overexpression leads to upregulation of SM22α (Chen S *et al., Nucl. Acids Res.* 31(4):1302-10 (2003); Ota T *et al., J. Cell Physiol.* 193(3):299-318 (2002)) |
| Vitamin D receptor | Vitamin D3 induces expression of TGFβ2; Smad3 binds and activates VDR (Yang L *et al., J. Cell Physiol.* 188(3):383-93 (2001); Aschenbrenner JK *et al., J. Surg. Res.* 100(2):171-5 (2001)) |
| Ephrin B2 | Ephrin A1 expression is decreased by ALK-5 overexpression (Ota T *et al., J. Cell Physiol.* 193(3):299-318 (2002)) |

EXAMPLE III

MULTI-ORGAN GENE EXPRESSION PROFILING OF CYNOMOLGUS MONKEY TREATED WITH FGF23CTP (100 μg/DAY SC)

[0085] *Introduction and summary.* The aim of this EXAMPLE was to identify for the peptide FGF23CTP modes of action with possible therapeutic applications by multi-organ microarray profiling in monkey. The peptide FGF23CTP (GPA006, GeneProt, Geneva,

Switzerland) is derived from a unique COOH-terminal domain of the FGF-23. It is a unique 75-mer COOH-terminal peptide of FGF-23 with no homology to regions of other FGF family members. See, PCT patent application WO 02/088,358, the contents of which are incorporated by reference. In the brain FGF-23 transcripts are preferentially expressed in the thalamus (Yamashita T *et al.*, *Biochem. Biophys. Res. Commu.*, 277: 494-8 (2000)). Mutations in this region of the FGF-23 molecule were proposed as causative events in a renal phosphate wasting syndrome responsible for a form of autosomal dominant rickets (Saito H et al., *Am. J. Pathol.* 156: 697-707 (2002), White KE *et al.*, *Kidney Int.* 60: 2079-86 (2001)). A similar paraneoplastic form of this syndrome was accompanied by ectopic expression of FGF-23 in the tumor tissue (Shimada T *et al.*, *Proc. Natl. Acad. Sci. USA* 98: 6500-5 (2001)). FGF-23 is expressed in the ventrolateral thalamic nucleus of the brain. FGF23CTP has been derived *in silico* as a possible processing product of the fibroblast growth factor FGF-23 known to be involved in renal phosphate wasting syndromes. It was hypothesized that the 75-mer peptide could affect phosphate homeostasis.

[0086] FGF23CTP was given two weeks subcutaneously to cynomolgus monkeys and was found to affect critical pathways of cell differentiation in a multi-organ gene expression profiling analysis with high density human microarray assays. Comparison of the expression changes in sixteen different organs indicated common transcript level changes for genes involved in cell to cell signaling of growth and lineage determination, in cell cycling and in amino acid and ion transport. Although the FGF23CTP domain is unique and not found in other FGF family members, transcript levels for components of the FGF signaling pathway are affected in several organs. Serum protein levels of circulating IGF2-binding protein were decreased in the treated animals.

[0087] Genes involved in angiogenesis/vasculogenesis were found in to be impacted in several organs. The effect of FGF23CTP on angiogenesis was confirmed in a hypoxic vascular retinopathy mouse model.

[0088] *Methods*. FGF23CTP was administered subcutaneously to cynomolgus monkeys for two weeks at a dose of 100 μg/day. At the end of the treatment period samples from all organs are subjected to snap freezing at necropsy and are analyzed with GeneChip® expression profiling.

[0089] Total RNA was extracted from these frozen tissues using TRIzol® reagent (Life Technologies) according to the manufacturer's instructions. Total RNA was quantified by the

absorbance at $\lambda = 260$ nm ($A_{260nm}$) and the purity is estimated by the ratio $A_{260nm}/A_{280nm}$. Integrity is checked by denaturing gel electrophoresis. RNA is stored at -80°C until analysis.

[0090] Good quality total RNA was used to synthesize double-stranded cDNA using the Superscript® Choice System (Technologies, Gaithersburg, Maryland USA). The cDNA was then *in vitro* transcribed (MEGAscript™ T7 Kit, Ambion) to form biotin labeled cRNA. Next, 12 to 15 µg of labeled cRNA was hybridized to the Affymetrix Human U95A Version 2 expression probe arrays for 16 hours at 45°C. Arrays are then washed according to the EukGE-WS2 protocol (Affymetrix), and stained with 10 µg/ml of streptavidin-phycoerythrin conjugate (Molecular Probes). The signal was antibody-amplified with 2 mg/ml acetylated BSA (Life Technologies, Gaithersburg, Maryland USA), 100 mM MES, 1 M [Na+], 0.05 % Tween 20, 0.005 % Antiofoam (Sigma), 0.1mg/ml goat IgG and 0.5 mg/ml biotinylated antibody and re-stained with the streptavidin solution. After washing, the arrays were scanned twice with the Gene Array® scanner (Affymetrix).

[0091] The expression level was estimated by averaging the differences in signal intensity measured by oligonucleotide pairs of a given probe (AvgDiff value). The image acquisition and numerical translation software used was the Affymetrix Microarray Suite version 4 (MAS4).

[0092] To identify genes that are impacted by treatment, the dataset is initially filtered to exclude in a first wave of analysis genes whose values are systematically in the lower expression ranges where the experimental noise is high (at least an AvgDiff value of 80 in a number of assays corresponding to the smallest number of replicas of any assay point). In a second round of selection a threshold t-test p-value (0.05) identifies genes with different values between treated and non-treated based on a two component error model (Global Error Model) and, where possible, with a stepdown correction for multi-hypothesis testing (Benjamini and Hochberg false discovery rate).

[0093] The selected genelists are then compared with established genelists for pathways and cellular components using Fisher's exact test. Venn diagrams are used to identify the gene changes that are in common between the different organs. Expression profiles of highly relevant genes are used to find genes with correlated changes at individual assay points, using several distance metrics (standard, Pearson).

[0094] The decision to consider a specific gene relevant is based on a conjunction of numerical changes identified by exploratory filtering and statistical algorithms as described above and the relationship to other modulated genes that point to a common biological theme.

[0095] A filter was applied on the FGF23CTP rostral hypothalamus expression raw data from a raw value of at least 80.0 in at least four out of eight conditions.

[0096] A selection was made on treatment conditions for FGF23CTP rostral hypothalamus based on statistically significant differences with a p-value cutoff 0.05 and a multiple testing correction based on the Benjamini and Hochberg False Discovery Rate. This restriction tested 5134 genes. About 5.0% of the identified genes would be expected to pass the restriction by chance.

[0097] *Results*. At the RNA level, it is clear that the compound affects key genes controlling cellular differentiation and proliferation, especially growth factors and growth factor receptors. Genes critically involved in angiogenesis are found in several organs. There is also a multi-organ effect on transcripts for components of the retinoblastoma cycling control checkpoint. The rostral hypothalamus shows the most pronounced changes especially for transport proteins and cytoarchitecture.

[0098] A cross comparison of organs analyzed reveals that FGF23CTP affects the same or closely related pathways and provokes similar cellular effects. No routine clinical or biochemical changes are observed in the treated animals. Surprisingly, no effect on phosphate metabolism is observed.

[0099] TABLES 3 to 6 show that at the RNA level FGF23CTP affects key genes controlling cellular differentiation and proliferation, especially growth factors and growth factor receptors (TABLE 3). Genes critically involved in angiogenesis are found to be altered in several organs upon treatment with FGF23CTP (TABLE 4). There is also a multi-organ effect on transcripts for components of the retinoblastoma cycling control checkpoint (TABLE 5). The rostral hypothalamus shows the most pronounced changes especially on transcripts corresponding to proteins involved in transport and cytoarchitecture (TABLE 6).

[00100] In particular, FGF23CTP affects several molecules that have been described to play a role in the pathogenesis of malignant proliferation of glial cells and precursors: Epidermal growth factor (EGF) (Hoi Sang U *et al., J. Neurosurg*. 82: 841-846 (1995); Wu CJ *et al., Oncogene* 19: 3999-4010 (2000)), Bax (Streffer JR *et al., J. Neurooncol*. 56: 43-49 (2002); Martin S *et al., J. Neurooncol*. 52: 129-139 (2001)), connexin 43 (Huang R *et al., Cancer Res*. 62: 2806-2812 (2002); Soroceanu L *et al., Glia* 33: 107-117 (2001)), PKR (Shir A. and Levitzki A., *Nature Biotechnology* 20: 895-900 (2002)), neurofibromin (NF1) (Cichowski K & Jacks T, *Cell* 104: 593-604 (2001); Gutmann, DH *et al., Hum. Mol. Genet*. 10: 3009-3016 (2001)).

[00101] *Growth factors and growth factor receptors*. Several families of growth factors and related growth factor receptors including fibroblast growth factor (FGF)-receptors and other important extracellular signaling molecules for cell differentiation and maintenance, like members of the bone morphogenetic proteins (BMP), transforming growth factor (TGF), insulin-like growth factor (IGF), tumor necrosis factor (TNF) families are found to be impacted in more than one organ by treatment with FGF23CTP (TABLE 3).

## TABLE 3
## Genes for Growth Factors and Receptors

| Gene name | Organ | Treatment group of 2 males and 2 females: Control — Gene expression level after 2 weeks (mean and range) | Treatment group of 2 males and 2 females: FGF23CTP — Gene expression level after 2 weeks (mean and range) |
|---|---|---|---|
| Activin A receptor type II | ROHY | 24.8 (20 to 32.6) | 54.9 (37.2 to 78.7) |
| | Adrenal | 20.6 (7.8 to 34.1) | 58.5 (34.3 to 72.8) |
| | Retina | 57.4 (48.2 to 70.6) | 34.1 (27.7 to 38) |
| Bone morphogenetic protein 10 | Kidney | 74.6 (68.9 to 79.7) | 138.6 (114.9 to 172.3) |
| | Liver | 96.4 (92.3 to 103.3) | 134.1 (108.8 to 152.6) |
| Bone morphogenetic protein 1 | Adrenal | 131.1 (81.5 to 150.6) | 210.6 (147.5 to 361.8) |
| Growth differentiation factor 1 | ROHY | 866 (753.5 to 911.2) | 1,410.2 (1,333.2 to 1,542.9) |
| Bone morphogenetic protein 2A | Duodenum | 121.3 (100.4 to 146.9) | 55.3 (37.9 to 80.1) |
| Bone morphogenetic protein receptor, type II | Adrenal | 64.6 (57.9 to 69.8) | 101.25 (71.2 to 156.5) |
| Colony-stimulating factor-1 | ROHY | 535.7 (470 to 598.2) | 802.5 (667.6 to 884.5) |
| | Kidney | 424.3 (316.1 to 576.8) | 659.75 (574.5 to 870.1) |
| | B marrow | 1,269.2 (934.5 to 1,493.8) | 981.3 (782.2 to 1,091.5) |
| Epidermal growth factor receptor | ROHY | 156.5 (138.4 to 167) | 103.1 (88.4 to 112.1) |
| EGF-response factor 1 | AV node | 1,141.3 (1,044.4 to 1,317.9) | 1,819.7(1,641.5 to 2,170.2) |
| EPHB2v protein-tyrosine kinase | ROHY | 173.5 (119.8 to 204.5) | 119.1 (113.2 to 121.8) |
| | Liver | 52.85 (35.2 to 64.7) | 82.6 (61.6 to 95.6) |
| FGF receptor-1 | ROHY | 669.9 (637 to 699.9) | 932.4 (782.6 to 1,125.6) |
| | Thymus | 224.5 (209.1 to 248.5) | 153.2 (133.2 to 169.6) |
| | Adrenal | 521.3(482.8 to 599) | 624.2 (559.4 to 721.9) |
| Farnesol receptor HRR-1 | Liver | 267.7 (259.6 to 276.4) | 383.3 (342.5 to 460.8) |
| | Duodenum | 28.3 (21.3 to 36.4) | 53.1 (44.6 to 67.1) |
| Insulin-like growth factor binding protein 2 | AV node | 1,371.2 (1,134 to 1,523.8) | 880.75 (707.4 to 1,226.9) |
| | Liver | 5'840.5 (4'269.2 to 6'849.6) | 3,858.6 (3,160.1 to 5'301.8) |
| | Retina | 1,396.8 (723.4 to 2,068.7) | 807.9 (506.4 to 1,143.3) |
| Insulin-like growth factor I | Muscle | 157.4 (115.9 to 179.9) | 107.65 (83.5 to 140.1) |
| | Liver | 649.675 (429.7 to 879.7) | 1,055.4 (838.1 to 1,331.6) |
| Insulin-like growth factor binding protein 1 | Liver | 3,238.1 (2,346.3 to 3,807.7) | 1,517 (105.7 to 2,449.4) |
| Insulin-like growth factor II | AV node | 401.275 (359.7 to 462.2) | 565.275 (524.7 to 619.9) |
| | Liver | 426.5 (401.1 to 482.9) | 606.9 (554 to 687.6) |
| | B Marrow | 295.4 (251.2 to 339.8) | 209.5 (183.5 to 236.8) |
| Neuregulin 1 | Kidney | 66.3 (42.7 to 105.8) | 150.725 (119.4 to 193.9) |
| | Hippocampus | 125.4 (105.1 to 133.3) | 86.075 (70.7 to 97.8) |
| Retinoid X receptor-beta | ROHY | 136.7 (120.7 to 154.4) | 106.925 (84.4 to 139.5) |
| Retinoid X receptor-gamma | Muscle | 253.925 (193.1 to 285.1) | 580.9 (391.3 to 704.5) |
| Transforming growth factor β 2 | Muscle | 20.85 (20 to 23.4) | 58.3 (39.3 to 66.9) |
| Transforming growth factor β 3 | ROHY | 37.9 (20 to 65.6) | 88.4 (77 to 104.1) |
| Tumor necrosis factor receptor-2 | ROHY | 70.3 (43.2 to 92.2) | 126.4 (117.8 to 136.4) |
| TNFR-related death receptor-6 | Re atrium | 100.6 (93.7 to 109.3) | 144.65 (123.9 to 175.5) |
| | ROHY | 155.1 (128.2 to 181.7) | 98.8 (69.1 to 137.3) |
| TNFR-related death receptor-3 | Muscle | 119.5 (92.9 to 133.6) | 82.4 (43.2 to 104.5) |

ROHY: rostral hypothalamus; B Marrow: bone marrow; AV node: heart atrioventricular (AV) node.

[00102] *Angiogenesis/vasculogenesis*. Transcript level changes for genes specifically involved in angiogenesis/vasculogenesis are found in several organs of animals treated with FGF23CTP (TABLE 4).

TABLE 4
Genes for Angiogenesis

| Gene name | Organ | Treatment group of 2 males and 2 females<br>Control<br>Gene expression level after 2 weeks (mean and range) | <br>FGF23CTP<br>Gene expression level after 2 weeks (mean and range) |
|---|---|---|---|
| Vascular endothelial growth factor-A (VEGFA) | ROHY | 491 (431.1 to 570.1) | 326.175 (309.6 to 366.3) |
| | AV node | 170.3 (106.6 to 234.9) | 363.7 (302.7 to 506.7) |
| VEGFB | ROHY | 484 (421.6 to 535.3) | 626.2 (527.4 to 776.7) |
| Placental growth factor | Muscle | 45.6 (20 to 78.4) | 91.4 (60.8 to 112.6) |
| FLT receptor tyrosine kinase 1 | Duodenum | 30.1 (20 to 60.5) | 70.7 (52.3 to 80.6) |
| | AV node | 56.5 (22.7 to 76.1) | 120.1 (77 to 156.4) |
| FLK1 receptor tyrosine kinase | Adrenal | 57.1 (49 to 62.5) | 42.7 (32.1 to 51) |
| Endothelial differentiation receptor 6 | Muscle | 258.6 (215.2 to 328) | 181.9 (167.9 to 199.7) |
| | AV node | 161.1 (111.6 to 188) | 306.9 (251.2 to 365.4) |
| Endothelial differentiation receptor 4 | Bone Marrow | 261.3 (214.2 to 356.9) | 494.8 (355.9 to 700.9) |
| | Liver | 279 (227.2 to 361) | 391.7 (347.7 to 453) |
| Brain-specific angiogenesis inhibitor 2 | Retina | 27.1 (20 to 38.2) | 153.2 (82.2 to 201) |
| Brain-specific angiogenesis inhibitor 1 (MAS5) | ROHY | 97.8 (82.8 to 118.8) | 225.1 (169.2 to 283.8) |
| VE-cadherin | Adrenal | 63.8 (46.7 to 86.3) | 110.2 (108 to 113.8) |
| Angiopoietin-1 | Bone Marrow | 198.6 (175.4 to 221.8) | 123.2 (106.3 to 151.1) |
| | ROHY | 235.7 (204.7 to 306.9) | 162.5 (154.4 to 179.6) |
| TEK tyrosine kinase, angiopoietin receptor | Bone Marrow | 91.8 (67.1 to 106.3) | 52.7 (33.3 to 63.7) |

ROHY: rostral hypothalamus; AV node: heart atrioventricular (AV) node.

[00103] *Cycling: Retinoblastoma checkpoint*. Transcript levels of genes involved in cell cycle control, especially those genes involved in the transition from the G1 to S-phase, are affected in several organs by treatment with FGF23CTP (TABLE 5). In particular, expression of those genes upstream or downstream of the retinoblastoma gene product (Rb) phosphorylation step, a major downstream control step for growth factor-induced proliferation, is altered. FGF23CTP also affects transcript levels for cyclin-dependent kinase 4 and cyclins D2, D3 and E2 involved in Rb phosphorylation. A second control level is also mobilized with cyclin kinase inhibitors like p19INK4D, p21CIP1 and p27Kip1. Also affected is the inhibitor of p53 and Rb, Mdm2. The targets of the retinoblastoma protein are also involved: E2F1, E2F2, E2F5 and their binding partner Dp-2.

TABLE 5
Cell Cycling

| Gene name | Organ | Treatment group of 2 males and 2 females<br>Control<br>Gene expression level after 2 weeks (mean and range) | FGF23CTP<br>Gene expression level after 2 weeks (mean and range) |
|---|---|---|---|
| Cyclin-dependent kinase 4 | ROHY | 266 (240.1 to 283.7) | 197.6 (167.6 to 231.7) |
| Cdk-inhibitor p57KIP2 (KIP2) | Duodenum | 233.7 (212.1 to 251.9) | 138.6 (114.9 to 172.3) |
| | Thymus | 312.7 (292 to 341.1) | 247.9 (227.3 to 284.2) |
| Cyclin A/CDK2-associated p19 (Skp1) | ROHY | 1349.8 (1,306.6 to 1,420.1) | 1069.6 (902.3 to 1,278) |
| Cyclin D2 | Retina | 210.2 (172.2 to 233.2) | 132 (96.2 to 171.5) |
| | ROHY | 231.8 (201.1 to 248.6) | 197.1 (182.7 to 203.9) |
| Cyclin D3 | Muscle | 182.4 (157.2 to 198.2) | 149.2 (135 to 162) |
| Cyclin-dependent kinase 5 | Hippocampus | 213.9 (172.3 to 254.7) | 118.7 (96.6 to 162.3) |
| Cyclin E2 | Bone Marrow | 407 (367.4 to 491.4) | 257 (175.2 to 294.8) |
| Cyclin I | Duodenum | 1109.9 (1035.5 to 1157.2) | 900.6 (758.7 to 1054.7) |
| E2F-1 | Kidney | 94 (51.9 to 150.9) | 198.5 (135.8 to 263) |
| E2F-4 | Muscle | 888 (765.7 to 981.2) | 643.8 (471.5 to 765.7) |
| | Hippocampus | 208.7 (185.7 to 221.5) | 153.4 (116.7 to 228.6) |
| | ROHY | 812 (748.4 to 859.5) | 245.9 (63.2 to 581.9) |
| | Kidney | 505.6 (432.1 to 574.8) | 741.6 (652.5 to 802.8) |
| E2F-5 | Adrenal | 53.9 (49.2 to 57.6) | 39.6 (33.5 to 45) |
| E2F dimerization partner 2 (DP2) | Bone Marrow | 2091 (1617.9 to 2735.1) | 1222 (1021.1 to 1387.7) |
| Mdm2 | Bone Marrow | 40.925 (33.4 to 44.3) | 20 |
| Cdk-inhibitor p19 | ROHY | 163.6 (148.6 to 194.9) | 267.8 (243.2 to 314.2) |
| Cdk-inhibitor 1A p21 (Cip1) | Adrenal | 106.9 (85 to 125.1) | 51.75 (20 to 96.8) |
| p53 binding protein | Muscle | 269.35 (173 to 374.7) | 138.7 (97.7 to 172) |
| | ROHY | 217.5 (210.1 to 226.3) | 154.7 (143.7 to 159.9) |
| Retinoblastoma 1 | ROHY | 255.9 (248.3 to 266.1) | 467.9 (361.1 to 570) |
| Retinoblastoma binding protein 4 | ROHY | 361.1 (283.4 to 398.7) | 164 (131.5 to 225.4) |
| S-phase response (cyclin-related) | ROHY | 231.5 (199.8 to 270.3) | 176 (159.8 to 205.6) |
| | Re atrium | 126.75 (100.7 to 146.9) | 159.1 (153.4 to 168.9) |
| RalGDS/AF-6 (inhibitor of Cyclin D1) | ROHY | 414.9 (390.1 to 439.7) | 300.5 (284 to 313.9) |
| | | 438.1 (392.6 to 520.8) | 354.5 (335.8 to 369.9) |

ROHY: rostral hypothalamus

[00104] *Rostral hypothalamus.* The rostral hypothalamus is the organ with the most pronounced changes in transcript levels in animals treated with FGF23CTP. TABLE 6 reflects genes of defined pathways and cellular actions with the most significant changes in this organ. In particular, effects on cytoarchitecture genes are especially pronounced in brain tissues.

## TABLE 6
## Rostral Hypothalamus Genes

| Gene name | Treatment group of 2 males and 2 females<br>Control<br>Gene expression level after 2 weeks<br>(mean and range) | FGF23CTP<br>Gene expression level after 2 weeks<br>(mean and range) |
|---|---|---|
| Receptors | | |
| Retinoic acid receptor | 31.2 (26.8 to 41) | 110 (100.9 to 122.8) |
| Cholecystokinin A receptor | 87.1 (66.6 to 119.4) | 196 (172.3 to 218.3) |
| Jagged 1 | 417.2 (402.1 to 437.7) | 70.3 (20 to 161.9) |
| Protein kinases | | |
| c-abl | 214.4 (187.3 to 252.5) | 119.4 (103.4 to 127) |
| c-yes | 116.65 (107.9 to 127.8) | 64.7 (50.8 to 77.4) |
| c-raf | 404.7 (370.8 to 458.7) | 327.5 (281.6 to 356.9) |
| Pim1 | 487.7 (432.9 to 557.6) | 327.5 (281.6 to 356.9) |
| FYN | 1,086.1 (1,018.4 to 1,172.4) | 958.1 (891.3 to 1,007.6) |
| PKR Interferon-inducible | 179.475 (162 to 192.7) | 128.25 (112.1 to 140.9) |
| TYRO3 | 90.45 (78.7 to 106.8) | 163.1 (134.7 to 179.5) |
| Axl | 122.8 (112.4 to 140) | 60.3 (34.7 to 89.4) |
| TrkB | 108.6 (87.5 to 127.9) | 40.8 (23.6 to 59.4) |
| Erk3 | 588.5 (551.5 to 679.4) | 409.5 (370.5 to 482.7) |
| Phosphoinositide 3-kinase | 90.3 (78.2 to 111.9) | 241.5 (215.7 to 289.2) |
| Glycogen synthase kinase 3 | 70.4 (66.3 to 75.3) | 155.5 (127.9 to 180.1) |
| JNK2 | 137.225 (122.2 to 159.8) | 79.9 (64 to 95.2) |
| Ribosomal protein kinase B (RSK-B) | 49.8 (35.4 to 74.5) | 120 (90.4 to 141.8) |
| Janus kinase 1 | 106.7 (88.9 to 136.3) | 43.3 (20 to 64.7) |
| SFRS protein kinase 2 | 396.1 (369.5 to 420.3) | 205.8 (180.3 to 249.6) |
| Mnb (Minibrain) | 567.4 (561.4 to 571.6) | 446 (410.2 to 477.2) |
| Other signaling molecules | | |
| Phosphoinositide-3-kinase, catalytic, delta polypeptide | 90.3 (78.2 to 111.9) | 241.5 (215.7 to 289.2) |
| RAS p21 protein activator | 243.9 (211.5 to 270) | 163 (139.4 to 198.5) |
| STAT1 | 182 (171.2 to 188.5) | 119.3 (110.6 to 134.5) |
| Calcineurin A1 | 410.5 (367.6 to 486.4) | 173.975 (114.5 to 239.7) |
| 14-3-3 protein tau | 2,110 (1,964.8 to 2,211.2) | 1,748.5 (1,649.5 to 1,852) |
| Neurofibromin | 78.6 (74.5 to 88.1) | 144 (126.4 to 155.8) |
| Phosphodiesterase 4B | 123 (110.1 to 133.3) | 60.6 (46.7 to 72.3) |
| Phospholipase C | 125.9 (107.4 to 136.2) | 72.1 (56.8 to 81) |
| Transcription factors | | |
| Hypoxia-inducible factor 1 alpha | 611.5 (515.7 to 745.1) | 286.5 (223 to 339.5).1) |
| CREB binding protein | 165.4 (160 to 176.9) | 93 (82.4 to 100) |
| Jun D | 4'882.5 (4'592.6 to 5'361.6) | 6'499.1 (6'353.9 to 6'745.1) |
| Inhibitor of DNA binding 4 | 140.3 (113.3 to 157.9) | 67.6 (55.9 to 72.7) |
| Bmi-1 homeobox gene repressor | 553.7 (513.6 to 599.8) | 374.7 (352.6 to 410.4) |

TABLE 6
Rostral Hypothalamus Genes

| Gene name | Treatment group of 2 males and 2 females<br>Control<br>Gene expression level after 2 weeks<br>(mean and range) | FGF23CTP |
|---|---|---|
| Solute transport | | |
| Ca2+-ATPase | 325.1 (274.6 to 401) | 174.4 (130.8 to 247.5) |
| Sodium/hydrogen exchanger | 1,062.4 (1,039.5 to 1,095.9) | 785.5 (743.4 to 851.2) |
| Anion exchanger | 627.1 (555.6 to 676.3) | 844.5 (760.8 to 916.2) |
| Na,K-ATPase | 2,032.2 (1,743.5 to 2,239.6) | 1,453.2 (1,246.3 to 1,736.8) |
| CMP-sialic acid transporter | 265.8 (241 to 329.6) | 135.075 (112.8 to 159.1) |
| Vesicle transport | | |
| RAB1 | 293.5 (246.9 to 320.3) | 143.9 (93.8 to 214.9) |
| RAB5 | 1,266.2 (1,146.6 to 1,349) | 809.8 (745.3 to 894) |
| RAB11A | 554.8 (544.8 to 565.4) | 344.9 (284.1 to 395.4) |
| RAB11B | 182.3 (153.2 to 207.4) | 71.2 (41.8 to 110.9) |
| RNP24 | 867.4 (809.5 to 903.7) | 558.2 (486.3 to 642.9) |
| SNARE | 140 (127.2 to 158.5) | 55.4 (41.2 to 69.7) |
| GDP dissociation inhibitor beta | 166.6 (156.5 to 173.4) | 125.8 (104.1 to 142.6) |
| NIPSNAP2 (glioblastoma co-amplified) | 212.9 (188.1 to 226.2) | 118.7 (88.9 to 172) |
| Apoptosis | | |
| Bcl-2-binding protein Nip3 | 1,099 (1,056.1 to 1,135.1) | 794.8 (709.9 to 841.2) |
| BAX | 30.95 (20 to 57.2) | 134.4 (75.2 to 171) |
| Cytoarchitecture | | |
| Tropomyosin 2 (beta) | 20 | 131.6 (112.4 to 150.2) |
| Myosin regulatory light chain | 328.5 (298.3 to 374) | 177.2 (153.5 to 214) |
| Tropomyosin 4 | 72 (54.7 to 86.6) | 140.6 (126.5 to 166.8) |
| Pinin, Desmosome associated protein | 181.7 (164 to 203.2) | 119.7 (108 to 131.5) |
| Connexin 43 | 410.4 (321.4 to 446.4) | 184.5 (132.2 to 265.8) |
| Delta 2 catenin | 239.6 (178.9 to 281.2) | 118 (103.9 to 146.9) |
| Reticulon 4 | 2,343,7 (2,019.1 to 2,516.7) | 1,309.2 (1,126.2 to 1,645.4) |
| Neural cell adhesion molecule 1 | 428 (297.1 to 524.2) | 988.6 (841.6 to 1,196) |
| Amyloid beta precursor protein (cytoplasmic tail)-binding protein 2 | 400 (351.1 to 435) | 191.3 (161.2 to 218.8) |
| Myelinization | | |
| Oligodendrocyte myelin glycoprotein OMGP | 1,144 (1,037.9 to 1,260.2) | 773.5 (619.7 to 986) |
| Myelin oligodendrocyte glycoprotein NPD | 176.8 (139.9 to 209.4) | 62.7 (25.9 to 80.7) |
| Sphingomyelinase | 585.5 (544.2 to 637.7) | 259.5 (229.5 to 281.4) |

[00105] As can be seen in the TABLE 7 (genelists per organ), there are genes representing these pathways with less pronounced changes in other organs. Effects on genes coding for transport molecules and intracellular signaling molecules are found in most organs. The effects on cytoarchitecture genes are especially pronounced in brain tissues. Shown in TABLE 7 are the Affymetrixs chip probeset ID, p-value, and gene description of the 602 selected genes.

## TABLE 7

## Gene Expression Changes at 4 Weeks in the Rostral Hypothalamus

| | | |
|---|---|---|
| 1 | 37316_r_at | 0.001189 Source: oy31e07.x1 Soares_parathyroid_tumor_NbHPA Homo sapiens cDNA clone IMAGE:1667460 3' similar to WP:F48E8.2 CE01954; mRNA sequence. |
| 2 | 32841_at | 0.001189 Source: Human nucleic acid binding protein gene, complete cds. |
| 3 | 32290_at | 0.005998 Source: Homo sapiens mRNA; cDNA DKFZp564L1916 (from clone DKFZp564L1916). |
| 4 | 38035_at | 0.005998 hMTMR6 |
| 5 | 38568_at | 0.005998 Source: Homo sapiens p53 binding protein mRNA, complete cds. |
| 6 | 33683_at | 0.005998 Source: Human mRNA for TI-227H. |
| 7 | 37995_s_at | 0.005998 Source: Human Fragile X mental retardation 1 FMR-1 gene, 3' end, clones BC72 and BC22. |
| 8 | 821_s_at | 0.005998 Source: Human folate receptor alpha (hFR) mRNA, partial cds. |
| 9 | 41562_at | 0.005998 putative |
| 10 | AFFX-HUMISGF3A/M97935_3_at | 0.005998 Source: Homo sapiens transcription factor ISGF-3 mRNA, complete cds. |
| 11 | 1132_s_at | 0.005998 Source: Homo sapiens retinoic acid receptor (gamma-7) mRNA. |
| 12 | 38363_at | 0.005998 Source: zd27g05.s1 Soares_fetal_heart_NbHH19W Homo sapiens cDNA clone IMAGE:341912 3' similar to PIR:S47066 S47066 zinc finger protein - human ;, mRNA sequence. |
| 13 | 32037_r_at | 0.005998 Source: Homo sapiens ribonuclease P protein subunit p14 (Rpp14) mRNA, complete cds. |
| 14 | 31879_at | 0.005998 Source: Human FUSE binding protein 3 (FBP3) mRNA, partial cds. |
| 15 | 38102_at | 0.005998 Source: 51f12 Human retina cDNA randomly primed sublibrary Homo sapiens cDNA, mRNA sequence. |
| 16 | 193_at | 0.005998 Source: Human transcriptional activation factor TAFII32 mRNA, complete cds. |
| 17 | 40246_at | 0.005998 sequences encoding: dlg homology repeats (DHR): DHR1 855..1118, DHR2 1143..1403, DHR3 1563..1826; SH3 domain 1935..2132; guanylate kinase-related domain 2331..2858 |
| 18 | 40453_s_at | 0.005998 member of the family of SR protein pre-mRNA splicing factors |
| 19 | 35247_at | 0.005998 Source: PT2.1_13_A09.r tumor2 Homo sapiens cDNA 3', mRNA sequence. |
| 20 | 39280_at | 0.005998 polyleucine rich |
| 21 | 36971_at | 0.005998 Similar to a C. elegans protein encoded in cosmid C27F2 (U40419) |
| 22 | 39628_at | 0.005998 Source: wb33e07.x1 NCI_CGAP_GC6 Homo sapiens cDNA clone IMAGE:2307492 3' similar to SW:RAB9_HUMAN P51151 RAS-RELATED PROTEIN RAB-9. :, mRNA sequence. |
| 23 | 41437_at | 0.005998 Source: Homo sapiens mRNA; cDNA DKFZp564F1123 (from clone DKFZp564F1123); partial cds. |
| 24 | 39167_r_at | 0.005998 Source: Human mRNA for collagen binding protein 2, complete cds. |
| 25 | 40467_at | 0.005998 cytochrome b small subunit of complex II (succinate-ubiquinone oxidoreductase) |
| 26 | 33831_at | 0.005998 transcriptional adaptor |
| 27 | 38694_at | 0.005998 Source: Homo sapiens mRNA for KIAA0738 protein, complete cds. |
| 28 | 34644_at | 0.005998 Source: Homo sapiens mRNA for beta 2-microglobulin, complete cds. |
| 29 | 34763_at | 0.005998 SMC family |
| 30 | 40128_at | 0.005998 similar to hypothetical protein L8167.6 of Saccharomyces cerevisiae. |
| 31 | 33109_f_at | 0.00617 putative |
| 32 | 36972_at | 0.007286 microsomal fraction |
| 33 | 36576_at | 0.007579 Source: Homo sapiens histone macroH2A1.2 mRNA, complete cds. |
| 34 | 36868_at | 0.007579 Source: Homo sapiens clone 23741 mRNA sequence. |
| 35 | 33359_at | 0.007579 Source: Homo sapiens mRNA for KIAA0768 protein, partial cds. |
| 36 | 35094_f_at | 0.007579 Source: Homo sapiens leukocyte immunoglobulin-like receptor-4 (LIR-4) mRNA, complete cds. |
| 37 | 40354_at | 0.007579 member of 110-kDa heat shock protein (hsp110) family |

## TABLE 7

## Gene Expression Changes at 4 Weeks in the Rostral Hypothalamus

| | | | |
|---|---|---|---|
| 38 | 38318_at | 0.00765 | Source: Homo sapiens mRNA; cDNA DKFZp586G051 (from clone DKFZp586G051). |
| 39 | 36864_at | 0.007879 | Source: Homo sapiens mRNA for Pex3 protein. |
| 40 | 32171_at | 0.008176 | HUMAN EUKARYOTIC TRANSLATION INITIATION FACTOR 5 |
| 41 | 34393_r_at | 0.008176 | Rab1, splice variant |
| 42 | 1117_at | 0.008176 | Source: Homo sapiens cytidine deaminase (CDA) mRNA, complete cds |
| 43 | 1191_s_at | 0.008176 | Source: Homo sapiens mRNA for 26S proteasome subunit p44.5, complete cds. |
| 44 | 33705_at | 0.008176 | putative |
| 45 | 37409_at | 0.008458 | similar to human serine kinase SRPK1, encoded by GenBank Accession Number U09564; specific for serine/arginine-rich splicing factors |
| 46 | 949_s_at | 0.008458 | proteasome (prosome, macropain) 26S subunit, ATPase, 6 |
| 47 | 35642_at | 0.008458 | mitochondrial outer membrane protein; binds to metaxin 1, a related protein that participates in protein import into mitochondria |
| 48 | 35784_at | 0.008458 | SNARE protein |
| 49 | 34383_at | 0.008458 | Source: Homo sapiens hUBP mRNA for ubiquitin specific protease, complete cds. |
| 50 | 36691_at | 0.008458 | Source: H. sapiens mRNA for glutamine transaminase K. |
| 51 | 36542_at | 0.008458 | Source: Homo sapiens sodium-hydrogen exchanger 6 (NHE-6) mRNA, nuclear gene encoding mitochondrial protein, complete cds. |
| 52 | 40631_at | 0.008458 | Source: Human mRNA for Tob, complete cds. |
| 53 | 40605_at | 0.008458 | sorting nexin 4 |
| 54 | 37350_at | 0.008704 | match: proteins Q99795 Q91665 Q91664 O09052 P78310 P97792 Q91667 O60939 P54900 Q62861 Q61148 O00426 P06907 P25189 Q92677 P20938 P27573 P10522 P37301 match: patented sequence I80040 supported by GENSCAN and FGENES |
| 55 | 38687_at | 0.009005 | Source: Homo sapiens mRNA; cDNA DKFZp566D193 (from clone DKFZp566D193); partial cds. |
| 56 | 36546_r_at | 0.009584 | Start codon is not identified. hg04325 cDNA clone for KIAA0542 has a 933-bp insertion between 3296-3297, and a 187-bp insertion between 3368-3369 of the sequence of KIAA0542 |
| 57 | 39767_at | 0.009584 | Source: Human mRNA for KIAA0002 gene, complete cds. |
| 58 | 36110_at | 0.009584 | Source: Homo sapiens GTP-binding protein (RAB5) mRNA, complete cds. |
| 59 | 38093_at | 0.009584 | Source: Human clone 23722 mRNA sequence. |
| 60 | 37826_at | 0.009584 | Source: Homo sapiens unknown mRNA, sequence. |
| 61 | 32232_at | 0.009584 | Source: Homo sapiens NADH-ubiquinone oxidoreductase subunit CI-SGDH mRNA, complete cds. |
| 62 | 39544_at | 0.009584 | Source: Human mRNA for KIAA0353 gene, partial cds. |
| 63 | 33399_at | 0.009602 | Source: zl43c04.s1 Soares_pregnant_uterus_NbHPU Homo sapiens cDNA clone IMAGE:504678 3', mRNA sequence. |
| 64 | 1085_s_at | 0.009679 | phospholipase C |
| 65 | 34931_at | 0.009679 | Source: Homo sapiens mRNA for KIAA0940 protein, complete cds. |
| 66 | 39099_at | 0.009679 | COPII component; isoform A |
| 67 | AFFX-hum_alu_at | 0.009806 | ***ALU WARNING: Human Alu-Sq subfamily consensus sequence. |
| 68 | 35734_at | 0.009806 | Source: wo97g09.x1 NCI_CGAP_Kid11 Homo sapiens cDNA clone IMAGE:2463328 3', mRNA sequence. |
| 69 | 995_g_at | 0.009839 | Source: H. sapiens hR-PTPu gene for protein tyrosine phosphatase. |
| 70 | 38040_at | 0.01011 | SF |
| 71 | 37705_at | 0.01011 | Source: Human (ard-1) mRNA, complete cds. |
| 72 | 40893_at | 0.010187 | Source: Homo sapiens ATP-specific succinyl-CoA synthetase beta subunit (SCS) mRNA, partial cds. |
| 73 | 36980_at | 0.010256 | Source: Human B4-2 protein mRNA, complete cds. |
| 74 | 1674_at | 0.010419 | cellular yes-1 protein |
| 75 | 39989_at | 0.010453 | Source: H. sapiens mRNA for ragB protein. |
| 76 | 34905_at | 0.010453 | Source: oq24f02.s1 NCI_CGAP_GC4 Homo sapiens cDNA clone IMAGE:1587291 3' similar to TR:Q62643 Q62643 GLUTAMATE RECEPTOR KA2 SUBUNIT. [1] ;, mRNA sequence. |

## TABLE 7

## Gene Expression Changes at 4 Weeks in the Rostral Hypothalamus

| | | |
|---|---|---|
| 77 | 31503_at | 0.010453 Source: 50h7 Human retina cDNA randomly primed sublibrary Homo sapiens cDNA, mRNA sequence. |
| 78 | 38808_at | 0.010453 Source: Human mRNA for Mr 110,000 antigen, complete cds. |
| 79 | 39923_at | 0.010453 Source: wo84c08.x1 NCI_CGAP_Kid11 Homo sapiens cDNA clone IMAGE:2462030 3', mRNA sequence. |
| 80 | 32695_at | 0.010685 isoform 2 match: protein Q99991 |
| 81 | 36224_g_at | 0.011036 Source: wf12b02.x1 Soares_NFL_T_GBC_S1 Homo sapiens cDNA clone IMAGE:2350347 3', mRNA sequence. |
| 82 | 36171_at | 0.011036 Source: th60h07.x1 NCI_CGAP_Ov23 Homo sapiens cDNA clone IMAGE:2122717 3' similar to SW:P15_HUMAN P53999 ACTIVATED RNA POLYMERASE II TRANSCRIPTIONAL COACTIVATOR P15 ;, mRNA sequence. |
| 83 | 39598_at | 0.011036 gap junction protein (aa 1-283) |
| 84 | 33543_s_at | 0.011036 desmosome associated protein; phosphoprotein |
| 85 | 429_f_at | 0.011036 Source: Human beta-tubulin gene (5-beta) with ten Alu family members. |
| 86 | 36326_at | 0.011036 Source: Human NSCL-2 gene sequence. |
| 87 | 35767_at | 0.011436 Source: tn20b01.x1 NCI_CGAP_Bm25 Homo sapiens cDNA clone IMAGE:2168137 3' similar to TR:O08765 O08765 GEF-2. ;, mRNA sequence. |
| 88 | 34819_at | 0.011497 Source: Human mRNA for MGC-24, complete cds. |
| 89 | 40861_at | 0.011497 Source: Human mRNA for KIAA0026 gene, complete cds. |
| 90 | 34302_at | 0.011497 Source: Human translation initiation factor eIF3 p44 subunit mRNA, complete cds. |
| 91 | 34677_f_at | 0.011497 Source: Homo sapiens mRNA for TL132. |
| 92 | 32744_at | 0.011881 Source: DU3.2-7.G08.r DU-145 Homo sapiens cDNA 5', mRNA sequence. |
| 93 | 38010_at | 0.012279 pro-apoptotic mitochondrial protein |
| 94 | 41651_at | 0.012367 Source: Homo sapiens mRNA for KIAA1033 protein, partial cds. |
| 95 | 36991_at | 0.012367 SR protein family member; SR domain: (bp. 583..1529); RNA binding domains: RNP-2 (bp. 57..80) and RNP-1 (bp. 150..173) |
| 96 | 33107_at | 0.012863 Source: Homo sapiens mRNA for KIAA0898 protein, partial cds. |
| 97 | 35297_at | 0.012932 Transcriptional coactivator P15 like |
| 98 | 35999_r_at | 0.012932 Source: Homo sapiens mRNA for KIAA0781 protein, partial cds. |
| 99 | 40864_at | 0.013006 Source: Homo sapiens mRNA, clone:PO2ST9. |
| 100 | 41178_at | 0.013006 Source: H. sapiens mRNA for ribosomal protein L11. |
| 101 | 1030_s_at | 0.013006 found in the camptothecin resistant clone CEM/C2 |
| 102 | 40854_at | 0.013006 core protein II precursor |
| 103 | 39382_at | 0.013006 Source: Homo sapiens mRNA for KIAA0517 protein, partial cds. |
| 104 | 32823_at | 0.013006 Source: 51a1 Human retina cDNA randomly primed sublibrary Homo sapiens cDNA, mRNA sequence. |
| 105 | 39748_at | 0.013006 Source: Homo sapiens mRNA; cDNA DKFZp564D016 (from clone DKFZp564D016). |
| 106 | 35005_at | 0.013006 Source: Homo sapiens type 6 nucleoside diphosphate kinase NM23-H6 (NM23-H6) mRNA, complete cds. |
| 107 | 32781_f_at | 0.013006 Source: zk65d06.r1 Soares_pregnant_uterus_NbHPU Homo sapiens cDNA clone IMAGE:487691 5', mRNA sequence. |
| 108 | 38705_at | 0.013006 Source: qo77c11.x1 NCI_CGAP_Kid5 Homo sapiens cDNA clone IMAGE:1914548 3' similar to gb:S57283 ENDOTHELIN B RECEPTOR PRECURSOR (HUMAN);, mRNA sequence. |
| 109 | 35838_at | 0.013006 similar to human zinc finger protein encoded by GenBank Accession Number M91592 |
| 110 | 185_at | 0.013006 paraneoplastic Ri antigen |
| 111 | 38738_at | 0.013006 ubiquitin-like protein |
| 112 | 36588_at | 0.013006 hk05647 cDNA clone for KIAA0810 has a 142-bp insertion at position 1028 of the sequence of KIAA0810. |
| 113 | 36596_r_at | 0.013006 This sequence comes from Fig. 4 |
| 114 | 1537_at | 0.013006 Source: Human mRNA for precursor of epidermal growth factor receptor. |
| 115 | 31881_at | 0.013154 Source: Homo sapiens mRNA for Hmob33 protein, 3' untranslated region. |
| 116 | 33440_at | 0.013327 Source: Human two-handed zinc finger protein ZEB mRNA, partial cds. |

## TABLE 7

## Gene Expression Changes at 4 Weeks in the Rostral Hypothalamus

| | | |
|---|---|---|
| 117 | 37359_at | 0.013357 Source: Human mRNA for KIAA0102 gene, complete cds. |
| 118 | 35681_r_at | 0.013357 Source: Homo sapiens mRNA for KIAA0569 protein, complete cds. |
| 119 | 34381_at | 0.013357 Source: as86g01.x1 Barstead colon HPLRB7 Homo sapiens cDNA clone IMAGE:2335632 3' similar to gb:X16560 CYTOCHROME C OXIDASE POLYPEPTIDE VIIC PRECURSOR (HUMAN);, mRNA sequence. |
| 120 | 192_at | 0.013357 Source: Human TFIID subunit TAFII55 (TAFII55) mRNA, complete cds. |
| 121 | 41547_at | 0.013381 amino terminus similar to protein encoded by GenBank Accession Number AF047473; structurally similar to Homo sapiens Rael protein encoded by GenBank Accession Number U84720; similar to Mus musculus protein encoded by GenBank Accession Number U67327; contains WD40 repeat motif, also termed tip-asp, G-beta repeat; binds C-terminal domain of BUB1 kinase in vitro |
| 122 | 31850_at | 0.013494 Source: Human gamma-glutamylcysteine synthetase (GCS) mRNA, complete cds. |
| 123 | 36660_at | 0.013702 GTPase |
| 124 | 40588_r_at | 0.013889 Source: Homo sapiens p18 protein mRNA, complete cds. |
| 125 | 36585_at | 0.014379 Source: Human ADP-ribosylation factor 4 (ARF4) mRNA, complete cds |
| 126 | 39388_at | 0.014587 Source: ok71f11.s1 NCI_CGAP_GC4 Homo sapiens cDNA clone IMAGE:1519437 3', mRNA sequence. |
| 127 | 38971_r_at | 0.014994 HIV-1, Nef-associated factor 1 beta |
| 128 | 32335_r_at | 0.015192 Source: Homo sapiens mRNA for polyubiquitin UbC, complete cds. |
| 129 | 725_i_at | 0.015192 |
| 130 | 40122_at | 0.015227 NS1-associated protein 1 |
| 131 | 38908_s_at | 0.015746 DNA polymerase zeta unspliced 3' region |
| 132 | 35709_at | 0.015746 Source: Homo sapiens clone 23923 mRNA sequence. |
| 133 | 31936_s_at | 0.015746 Start codon is not identified. hh01734 cDNA clone for KIAA0430 has a 61-bp deletion at the region from 3167 to 3227 of the sequence of KIAA0430 |
| 134 | 40146_at | 0.016164 Human mRNA for Rap1B protein |
| 135 | 34231_at | 0.016164 binds the large subunit (ORC1L) of the origin recognition complex (ORC) |
| 136 | 38375_at | 0.016327 Source: Homo sapiens esterase D mRNA, complete cds. |
| 137 | 35727_at | 0.016387 Source: qj64d06.x1 NCI_CGAP_Kid3 Homo sapiens cDNA clone IMAGE:1864235 3' similar to WP:F19B6.1 CE05666 URIDINE KINASE ;, mRNA sequence. |
| 138 | 34532_at | 0.016387 Source: Homo sapiens clone 23705 mRNA sequence. |
| 139 | 35969_at | 0.016529 Source: yx67h12.s1 Soares melanocyte 2NbHM Homo sapiens cDNA clone IMAGE:266855 3', mRNA sequence. |
| 140 | 41635_at | 0.017145 Source: Human mRNA for KIAA0105 gene, complete cds. |
| 141 | 35221_at | 0.017213 Source: H. sapiens mRNA for pur alpha extended 3'untranslated region. |
| 142 | 33456_at | 0.017213 Source: Homo sapiens EB1 mRNA, complete cds. |
| 143 | 41866_s_at | 0.017213 member of the dystrophin gene family |
| 144 | 39733_at | 0.017293 similar to Homo sapiens KIA0025 product encoded by GenBank Accession Number D14695 |
| 145 | 35826_at | 0.017369 Source: Homo sapiens transcription factor Tat-CT1 mRNA, complete cds. |
| 146 | 33178_at | 0.017509 similar to R. norvegicus Jagged1 protein |
| 147 | 35329_at | 0.017509 similar to Saccharomyces cerevisiae ORF YIL043C/CBR1/CBR5/CBR |
| 148 | 35271_at | 0.017509 one of seven subunits of the Arp2/3 protein complex; actin-related protein |
| 149 | 39722_at | 0.017509 Source: Homo sapiens nuclear receptor co-repressor N-CoR mRNA, complete cds. |
| 150 | 39740_g_at | 0.017509 Source: Homo sapiens alpha NAC mRNA, complete cds. |
| 151 | 38086_at | 0.017509 Source: Homo sapiens mRNA for KIAA0466 protein, partial cds. |
| 152 | 940_g_at | 0.017509 neurofibromin 1 (neurofibromatosis, von Recklinghausen disease, Watson disease) |
| 153 | 37928_at | 0.017509 Source: af53a04.s1 Soares_total_fetus_Nb2HF8_9w Homo sapiens cDNA clone IMAGE:1035342 3' similar to gb:X59710 CCAAT-BINDING TRANSCRIPTION FACTOR SUBUNIT A (HUMAN);, mRNA sequence. |
| 154 | 40852_at | 0.017509 similar to Drosophila tudor |

## TABLE 7

## Gene Expression Changes at 4 Weeks in the Rostral Hypothalamus

| | | |
|---|---|---|
| 155 | 35786_at | 0.017509 Source: Homo sapiens mRNA for KIAA0476 protein, complete cds. |
| 156 | 1039_s_at | 0.017509 basic helix-loop-helix transcription factor |
| 157 | 37010_at | 0.017509 Source: qf76b12.x1 Soares_fetal_lung_NbHL19W Homo sapiens cDNA clone IMAGE:1755935 3' similar to SW:T2AG_HUMAN P52657 TRANSCRIPTION INITIATION FACTOR IIA GAMMA CHAIN ;, mRNA sequence. |
| 158 | 1772_s_at | 0.017509 Source: Human farnesyl-protein transferase alpha-subunit mRNA, complete cds. |
| 159 | 38485_at | 0.017509 Source: nz14h07.s1 NCI_CGAP_GCB1 Homo sapiens cDNA clone IMAGE:1287805 3', mRNA sequence. |
| 160 | 32752_at | 0.017509 Source: zd65e10.s1 Soares_fetal_heart_NbHH19W Homo sapiens cDNA clone IMAGE:345546 3' similar to SW:N4AM_BOVIN Q05752 NADH-UBIQUINONE OXIDOREDUCTASE SUBUNIT B14.5A ;, mRNA sequence. |
| 161 | 37900_at | 0.017509 membrane protein |
| 162 | 38648_at | 0.017682 contains leucine repeat |
| 163 | 33438_at | 0.0178 Source: Homo sapiens mRNA; cDNA DKFZp564D012 (from clone DKFZp564D012). |
| 164 | 38277_at | 0.0178 calcineurin A1 |
| 165 | 39601_at | 0.0178 RDA32; similar to norel |
| 166 | 36641_at | 0.0178 similar to chicken alpha2 isoform |
| 167 | 33873_at | 0.017874 Nuclear protein with DNA-binding ability |
| 168 | 37740_r_at | 0.018039 ADP/ATP carrier protein |
| 169 | 32210_at | 0.018039 Source: Human phosphoglucomutase 1 (PGM1) mRNA, complete cds. |
| 170 | 32998_at | 0.019081 Source: Human cholecystokinin A receptor mRNA, complete cds |
| 171 | 38470_i_at | 0.019104 Source: Human mRNA for KIAA0228 gene, partial cds. |
| 172 | 36627_at | 0.019304 Source: H. sapiens mRNA for hevin like protein. |
| 173 | 32183_at | 0.019304 Gene product is 54 kDa but migrates aberrantly on SDS gels as a 70 kDa protein.; In mammalian cells, the protein colocalizes with many components of the pre-mRNA splicing machinery in the nucleus. |
| 174 | 32597_at | 0.019304 Source: H. sapiens p130 mRNA for 130K protein. |
| 175 | 33912_at | 0.019346 Source: Homo sapiens mRNA for farnesylated-proteins converting enzyme 1. |
| 176 | 36112_r_at | 0.019346 Source: H. sapiens PR264 gene. |
| 177 | 40125_at | 0.019346 Source: Homo sapiens integral membrane protein, calnexin, (IP90) mRNA, complete cds. |
| 178 | 1446_at | 0.019389 proteasome subunit C3 |
| 179 | 38702_at | 0.019389 Source: Homo sapiens clone 24781 mRNA sequence. |
| 180 | 39762_at | 0.019389 Source: Homo sapiens KIAA0425 mRNA, complete cds. |
| 181 | 36860_at | 0.01939 Source: Homo sapiens mRNA for KIAA1064 protein, partial cds. |
| 182 | 409_at | 0.01939 Source: Human mRNA for 14.3.3 protein, a protein kinase regulator. |
| 183 | 731_f_at | 0.019479 |
| 184 | 31526_f_at | 0.019479 Source: H. sapiens mRNA for tre oncogene (clone 213). |
| 185 | 36975_at | 0.019479 Source: 34d2 Human retina cDNA randomly primed sublibrary Homo sapiens cDNA, mRNA sequence. |
| 186 | 41418_at | 0.019479 Source: Homo sapiens mRNA for KIAA0821 protein, complete cds. |
| 187 | 38542_at | 0.019479 Source: Homo sapiens nucleophosmin phosphoprotein (NPM) gene, 3' flanking sequence. |
| 188 | 37994_at | 0.019479 unnamed protein product |
| 189 | 32574_at | 0.019479 Source: H. sapiens mRNA for sphingomyelinase. |
| 190 | 33094_s_at | 0.019479 Source: wq32a03.x1 NCI_CGAP_GC6 Homo sapiens cDNA clone IMAGE:2472940 3' similar to TR:Q13515 Q13515 BEADED FILAMENT PROTEIN CP49. ;, mRNA sequence. |
| 191 | 36408_at | 0.019479 Source: Homo sapiens ORCTL4 mRNA for organic-cation transporter like 4, complete cds. |
| 192 | 33740_at | 0.019673 Source: Homo sapiens clk2 kinase (CLK2), propin1, cote1, glucocerebrosidase (GBA), and metaxin genes, complete cds; metaxin pseudogene and glucocerebrosidase pseudogene; and thrombospondin3 (THBS3) gene, partial cds. |
| 193 | 40074_at | 0.020159 precursor polypeptide (AA -29 to 315) |

## TABLE 7
## Gene Expression Changes at 4 Weeks in the Rostral Hypothalamus

| | | |
|---|---|---|
| 194 | 34829_at | 0.020183 nucleolar protein; similar to yeast Cb5p |
| 195 | 41474_at | 0.020183 Source: H. sapiens mRNA for kinesin-2. |
| 196 | 35355_at | 0.020183 Source: Homo sapiens mRNA for KIAA0890 protein, complete cds. |
| 197 | 39677_at | 0.020183 Source: Human mRNA for KIAA0186 gene, complete cds. |
| 198 | 39346_at | 0.020223 Source: Human p62 mRNA, complete cds. |
| 199 | 1448_at | 0.020223 proteasome subunit C8 |
| 200 | 32710_at | 0.020223 Source: H. sapiens mRNA for voltage gated potassium channels, beta subunit. |
| 201 | 887_at | 0.020223 ORF |
| 202 | 33847_s_at | 0.020223 Source: qo19f03.x1 NCI_CGAP_Lu5 Homo sapiens cDNA clone IMAGE:1908989 3', mRNA sequence. |
| 203 | 38431_at | 0.020243 Source: Human protein kinase (JNK2) mRNA, complete cds. |
| 204 | 37785_at | 0.020243 Source: U69563 Soares infant brain 1NIB Homo sapiens cDNA clone 25050, mRNA sequence. |
| 205 | 39856_at | 0.020243 Source: at02f03.x1 Barstead aorta HPLRB6 Homo sapiens cDNA clone IMAGE:2353949 3' similar to gb:M15661 60S RIBOSOMAL PROTEIN L44 (HUMAN);, mRNA sequence. |
| 206 | 766_at | 0.020243 Source: Homo sapiens mRNA for galectin-9 isoform, complete cds. |
| 207 | 37569_at | 0.020243 Source: Homo sapiens calcium binding protein (ALG-2) mRNA, complete cds. |
| 208 | 34312_at | 0.020243 Source: oy33a12.x1 Soares_parathyroid_tumor_NbHPA Homo sapiens cDNA clone IMAGE:1667614 3', mRNA sequence. |
| 209 | 40083_at | 0.020243 Source: Homo sapiens mRNA for KIAA0625 protein, partial cds. |
| 210 | 40832_s_at | 0.020283 strong similarity to rat lamina associated polypeptide 1C |
| 211 | 1696_at | 0.020317 Source: Human mRNA for DNA polymerase beta, complete cds. |
| 212 | 39136_at | 0.020317 Source: Homo sapiens mRNA for oxidative-stress responsive 1, complete cds. |
| 213 | 34808_at | 0.020317 Source: Homo sapiens mRNA for KIAA0999 protein, partial cds. |
| 214 | 33433_at | 0.020449 Source: Homo sapiens mRNA; cDNA DKFZp564F0522 (from clone DKFZp564F0522). |
| 215 | 1663_at | 0.020494 |
| 216 | 32803_at | 0.020494 expression elevated in dividing 786-0 cells compared with confluent 786-0 cells |
| 217 | 34454_r_at | 0.020507 apolipoprotein |
| 218 | 33247_at | 0.020554 human homolog of fission yeast pad1 |
| 219 | 41606_at | 0.020554 developmentally regulated |
| 220 | 34478_at | 0.020641 Source: H. sapiens YPT3 mRNA. |
| 221 | 34306_at | 0.020641 Source: Homo sapiens KIAA0428 mRNA, complete cds. |
| 222 | 36295_at | 0.020641 Source: Human zinc finger protein ZNF134 mRNA, complete cds. |
| 223 | 35656_at | 0.020641 Source: Homo sapiens mRNA for RING-H2 protein RNF6, alternative exon 1a. |
| 224 | 36935_at | 0.020832 ras p21 GTP-ase-activating protein (GAP) |
| 225 | 37620_at | 0.020832 TAFII20; contains homology to histone H2B; TFIID subunit |
| 226 | 1848_at | 0.020832 Source: Human ras-related protein (Krev-1) mRNA, complete cds. |
| 227 | 35818_at | 0.021007 Source: Homo sapiens mRNA for cytochrome c, partial cds. |
| 228 | 39180_at | 0.021107 translocated in liposarcoma |
| 229 | 39792_at | 0.021292 hnRNP R |
| 230 | 34063_at | 0.021292 Source: Homo sapiens RecQ5 mRNA for DNA helicase, complete cds. |
| 231 | 1998_i_at | 0.021292 this sequence is a new splice variant called BAX delta in which exon 3 is deleted and exons 2 and 4 are spliced to each other |
| 232 | 33614_at | 0.021292 unnamed protein product |
| 233 | 36456_at | 0.021292 similarity to A. thaliana PRT1 |
| 234 | 36926_at | 0.021292 Source: H. sapiens ERK3 mRNA. |
| 235 | 33849_at | 0.021292 Source: Human pre-B cell enhancing factor (PBEF) mRNA, complete cds. |

## TABLE 7

## Gene Expression Changes at 4 Weeks in the Rostral Hypothalamus

| | | |
|---|---|---|
| 236 | 32624_at | 0.021292 strong similarity to rat tulip 1/2 |
| 237 | 38974_at | 0.021292 Source: Homo sapiens RNA-binding protein regulatory subunit mRNA, complete cds. |
| 238 | 36754_at | 0.021292 Source: H. sapiens gene PACAP for pituitary adenylate cyclase activating polypeptide. |
| 239 | 40637_at | 0.021292 Source: Human hsc70 gene for 71 kd heat shock cognate protein. |
| 240 | 32565_at | 0.021292 Unlike BAF60a and BAF60b this gene is highly expressed in muscle cells; similar to human BAF60a, BAF60b, BAF60c and yeast SWP73 and SWP73b |
| 241 | 36822_at | 0.021292 putative RNA binding protein; similar to human FUS/TLS and EWS |
| 242 | 38099_r_at | 0.021356 Source: Homo sapiens acyl-CoA synthetase 4 (ACS4) mRNA, complete cds. |
| 243 | 31536_at | 0.021356 Source: Homo sapiens mRNA for KIAA0886 protein, complete cds. |
| 244 | 36196_at | 0.021356 Source: Human phosphofructokinase (PFKM) mRNA, complete cds. |
| 245 | 36459_at | 0.021356 Source: Homo sapiens mRNA for KIAA0879 protein, complete cds. |
| 246 | 40423_at | 0.021356 Source: Homo sapiens mRNA for KIAA0903 protein, partial cds. |
| 247 | 32879_at | 0.021356 Source: Homo sapiens mRNA; cDNA DKFZp586L111 (from clone DKFZp586L111). |
| 248 | 39389_at | 0.021356 Source: Human CD9 antigen mRNA, complete cds. |
| 249 | 33368_at | 0.021356 Source: H. sapiens mRNA for Lon protease-like protein. |
| 250 | 41127_at | 0.021356 2 potential N-linked glycosylation sites at residues 201 and 206; in-frame stop codon 24 bp upstream of initiator methionine |
| 251 | 38443_at | 0.021356 Source: Human clone 23721 mRNA sequence. |
| 252 | 297_g_at | 0.021356 |
| 253 | 37392_at | 0.021356 beta subunit |
| 254 | 32205_at | 0.021356 PKR interacting protein |
| 255 | 32665_at | 0.02145 Source: Homo sapiens mRNA for protein phosphatase 2C (beta). |
| 256 | 39018_at | 0.02145 GSH-transferase; GSH-peroxidase |
| 257 | 35839_at | 0.021826 Source: Homo sapiens mRNA for squalene epoxidase, complete cds. |
| 258 | 32215_i_at | 0.021876 Source: Homo sapiens mRNA for KIAA0878 protein, complete cds. |
| 259 | 329_s_at | 0.022103 |
| 260 | 33027_at | 0.022103 Source: 39d11 Human retina cDNA randomly primed sublibrary Homo sapiens cDNA, mRNA sequence. |
| 261 | 381_s_at | 0.022103 Source: H. sapiens mRNA for phosphoinositide 3-kinase. |
| 262 | 38972_at | 0.022103 Source: Homo sapiens clone 24775 mRNA sequence. |
| 263 | 37668_at | 0.022175 Source: Human pre-mRNA splicing factor SF2p32, complete sequence. |
| 264 | 37868_s_at | 0.022178 MOG-25.6kD |
| 265 | 38809_s_at | 0.022233 Source: Homo sapiens mRNA for KIAA0519 protein, complete cds. |
| 266 | 810_at | 0.022455 similar to Dbl exchange factor and to pleckstrin |
| 267 | 1612_s_at | 0.022455 Source: Human junD mRNA. |
| 268 | 36981_at | 0.022455 Source: Homo sapiens clone 24452 mRNA sequence. |
| 269 | 40570_at | 0.022455 Source: Homo sapiens forkhead protein (FKHR) mRNA, complete cds. |
| 270 | 36536_at | 0.022475 Source: Homo sapiens clone 24732 unknown mRNA, partial cds. |
| 271 | 38801_at | 0.022475 Source: wg46h09.x1 Soares_NSF_F8_9W_OT_PA_P_S1 Homo sapiens cDNA clone IMAGE:2368193 3' similar to TR:O75453 O75453 VAMP-ASSOCIATED PROTEIN OF 33 KDA. ;, mRNA sequence. |
| 272 | 35777_at | 0.022475 Source: Homo sapiens mRNA for zinc finger protein, complete cds, clone: RES4-26. |
| 273 | 38254_at | 0.022475 Source: Homo sapiens mRNA for KIAA0882 protein, partial cds. |
| 274 | 38075_at | 0.022475 Source: H. sapiens h-Sp1 mRNA. |
| 275 | 34192_at | 0.022475 Source: Homo sapiens mRNA for KIAA0532 protein, partial cds. |
| 276 | 41212_r_at | 0.022485 Source: Human mRNA for KIAA0038 gene, partial cds. |
| 277 | 31510_s_at | 0.023142 Source: H. sapiens hH3.3B gene for histone H3.3. |

## TABLE 7

## Gene Expression Changes at 4 Weeks in the Rostral Hypothalamus

| | | |
|---|---|---|
| 278 | 32667_at | 0.023142 Source: Human alpha-5 collagen type IV (COL4A5) mRNA, 3' end. |
| 279 | 41536_at | 0.023264 match: proteins P47928 P41139 Q13005 |
| 280 | 34387_at | 0.023264 similar to putative product coded in C. elegans cosmid C01C10. |
| 281 | 36042_at | 0.023264 Source: H. sapiens trkB mRNA for protein-tyrosine kinase. |
| 282 | 38357_at | 0.023264 Source: Homo sapiens mRNA; cDNA DKFZp564D156 (from clone DKFZp564D156). |
| 283 | 36118_at | 0.023264 Source: Homo sapiens mRNA for steroid receptor coactivator 1e. |
| 284 | 34680_s_at | 0.023336 Source: Human mRNA for KIAA0107 gene, complete cds. |
| 285 | 31962_at | 0.023449 Source: Homo sapiens ribosomal protein L37a (RPL37A) mRNA, complete cds. |
| 286 | 33158_at | 0.023449 Source: Homo sapiens Kallmann syndrome (KAL) mRNA, complete cds. |
| 287 | 35184_at | 0.023449 Source: Homo sapiens mRNA for KIAA0546 protein, partial cds. |
| 288 | 35083_at | 0.023449 similar to SW:GOLI_DROME Q06003 GOLIATH PROTEIN |
| 289 | 32531_at | 0.023449 gap junction protein (AA 1-382) |
| 290 | 37038_at | 0.023449 Source: H. sapiens PXMP1 gene, exon 1 (and joined CDS). |
| 291 | 35750_at | 0.023449 Source: Homo sapiens mRNA; cDNA DKFZp564K0222 (from clone DKFZp564K0222). |
| 292 | 41823_at | 0.023581 Source: Homo sapiens mRNA for staufen protein, partial. |
| 293 | 1970_s_at | 0.023697 Source: H. sapiens FGFR2 mRNA. |
| 294 | 36488_at | 0.023982 Source: Homo sapiens mRNA for MEGF9, partial cds. |
| 295 | 33666_at | 0.024001 C protein |
| 296 | 39150_at | 0.024001 Source: U69559 Soares infant brain 1NIB Homo sapiens cDNA clone 26077, mRNA sequence. |
| 297 | 471_f_at | 0.024001 class III isotype; beta-3 |
| 298 | 33118_at | 0.024016 match to U03056 (PID:g532974); H_LUCA14.3 |
| 299 | 36988_at | 0.024016 Source: Human B12 protein mRNA, complete cds. |
| 300 | 32039_at | 0.024096 similar to cerebellar degeneration antigen beta-NAP and to the beta-1-adaptin and beta-2-adaptin subunits of clathrin-associated complexes |
| 301 | 37040_at | 0.024109 The ha1225 gene product is related to human alpha-glucosidase. |
| 302 | 36845_at | 0.024145 The KIAA0136 gene product is novel. |
| 303 | 1636_g_at | 0.024172 ABL is the cellular homolog proto-oncogene of Abelson's murine leukemia virus and is associated with the t9:22 chromosomal translocation with the BCR gene in chronic myelogenous and acute lymphoblastic leukemia; alternative splicing using exon 1a |
| 304 | 38710_at | 0.024734 Source: Homo sapiens mRNA; cDNA DKFZp564E242 (from clone DKFZp564E242). |
| 305 | 37914_at | 0.024734 Source: Human mRNA for KIAA0305 gene, complete cds. |
| 306 | 34355_at | 0.024895 Source: Homo sapiens mRNA for methyl-CpG-binding protein 2. |
| 307 | 1311_at | 0.024965 Source: Human mRNA for proteasome subunit HsN3, complete cds. |
| 308 | 35294_at | 0.024965 ribonucleoprotein autoantigen 60 kd subunit |
| 309 | 32313_at | 0.025167 fibroblast tropomyosin |
| 310 | 33082_at | 0.025167 Source: Homo sapiens integrin subunit alpha 10 precursor, mRNA, complete cds. |
| 311 | 33821_at | 0.025167 match: proteins: Tr:Q9Y3M0 Tr:Q9WU14 Sw:P39540 Tr:Q9Y396 |
| 312 | 35232_f_at | 0.025167 Source: oz26h05.x1 Soares_total_fetus_Nb2HF8_9w Homo sapiens cDNA clone IMAGE:1676505 3' similar to SW:CATR_GIALA Q24956 CALTRACTIN ;, mRNA sequence. |
| 313 | 37663_at | 0.025167 Source: Homo sapiens DDX1 gene, complete CDS. |
| 314 | 612_s_at | 0.025167 2',3'-cyclic-nucleotide 3'-phosphodiesterase (EC 3.1.4.37) |
| 315 | 36164_at | 0.025167 similar to the Rattus norvegicus dihydrolipoamide acetyltransferase component (E2) of pyruvate dehydrogenase complex Swiss Prot Accession Number P08461 |
| 316 | 38805_at | 0.025167 Source: H. sapiens mRNA for TGIF protein. |
| 317 | 40770_f_at | 0.025182 heterogeneous nuclear ribonucleoprotein D-like |

## TABLE 7

## Gene Expression Changes at 4 Weeks in the Rostral Hypothalamus

| | | |
|---|---|---|
| 318 | 34304_s_at | 0.025556 Source: Homo sapiens mRNA; cDNA DKFZp586G1923 (from clone DKFZp586G1923). |
| 319 | 34783_s_at | 0.025678 amino terminus similar to protein encoded by GenBank Accession Number AF047472; structurally similar to Homo sapiens Rael protein encoded by GenBank Accession Number U84720; similar to Mus musculus protein encoded by GenBank Accession Number U67327; contains WD40 repeat motif, also termed trp-asp, G-beta repeat; binds BUB1 kinase |
| 320 | 37575_at | 0.025678 Source: Homo sapiens mRNA; cDNA DKFZp586C1723 (from clone DKFZp586C1723). |
| 321 | 39755_at | 0.025678 match: proteins: Sw:P17861 Tr:O35426 |
| 322 | 37307_at | 0.025678 G protein alpha-subunit (AA 1-355) |
| 323 | 36946_at | 0.025684 human homolog of Drosophila mnb (minibrain) gene |
| 324 | 34425_at | 0.025684 does not contain alpha 3 domain |
| 325 | 40347_at | 0.025788 Source: ol39a08.s1 Soares_NFL_T_GBC_S1 Homo sapiens cDNA clone IMAGE:1525814 3' similar to contains element L1 repetitive element ;, mRNA sequence. |
| 326 | 32402_s_at | 0.025992 Source: H. sapiens mRNA for symplekin. |
| 327 | 32235_at | 0.026262 Source: Homo sapiens mRNA for KIAA0544 protein, partial cds. |
| 328 | 41063_g_at | 0.02649 Source: zc52c04.r1 Soares_senescent_fibroblasts_NbHSF Homo sapiens cDNA clone IMAGE:325926 5' similar to SW:BMI1_MOUSE P25916 DNA-BINDING PROTEIN BMI-1. [1] ;, mRNA sequence. |
| 329 | 33373_at | 0.026577 Source: Homo sapiens mRNA; cDNA DKFZp564O0122 (from clone DKFZp564O0122). |
| 330 | 34647_at | 0.026577 p68 protein (AA 1-614) |
| 331 | 32240_at | 0.026673 Source: Human mRNA for KIAA0072 gene, partial cds. |
| 332 | 37324_at | 0.026673 put. transferrin receptor (aa 1-760) |
| 333 | 36337_at | 0.02682 Source: wi67f11.x1 NCI_CGAP_Kid12 Homo sapiens cDNA clone IMAGE:2398413 3' similar to TR:O75257 O75257 R31180_1. ;contains TAR1.t1 TAR1 repetitive element ;, mRNA sequence. |
| 334 | 33447_at | 0.02682 myosin regulatory light chain |
| 335 | 39373_at | 0.026839 Source: Homo sapiens clone 23716 mRNA sequence. |
| 336 | 32844_at | 0.026839 eIF4GI |
| 337 | 31509_at | 0.026839 Source: H. sapiens BBC1 mRNA. |
| 338 | 35246_at | 0.026839 Source: Human receptor tyrosine kinase (DTK) mRNA, complete cds. |
| 339 | 31853_at | 0.026839 polycomb-group protein; homolog of Drosophila esc (extra sex combs); EED |
| 340 | 37619_at | 0.026839 KIAA0094 gene product is related to S.cerevisiae methionine aminopeptidase. |
| 341 | 35790_at | 0.026839 Source: Homo sapiens H beta 58 homolog mRNA, complete cds. |
| 342 | 32730_at | 0.027032 Source: Homo sapiens mRNA; cDNA DKFZp564H142 (from clone DKFZp564H142). |
| 343 | 226_at | 0.027032 Source: Human cAMP-dependent protein kinase type I-alpha subunit (PRKAR1A) mRNA, complete cds. |
| 344 | 37424_at | 0.027032 a-helix coiled-coil rod homologue |
| 345 | 38982_at | 0.027032 Source: 53g9 Human retina cDNA randomly primed sublibrary Homo sapiens cDNA, mRNA sequence. |
| 346 | 32504_at | 0.027032 Source: wu69c05.x1 NCI_CGAP_Kid3 Homo sapiens cDNA clone IMAGE:2525288 3', mRNA sequence. |
| 347 | 41463_at | 0.027032 Source: DKFZp434B0222_s1 434 (synonym: htes3) Homo sapiens cDNA clone DKFZp434B0222 3', mRNA sequence. |
| 348 | 35759_at | 0.027088 similar to mouse chaperonin-containing TCP-1 beta subunit |
| 349 | 168_at | 0.027139 Source: Human adenosine kinase mRNA, complete cds. |
| 350 | 35140_at | 0.027139 Source: yh11b03.s1 Soares infant brain 1NIB Homo sapiens cDNA clone IMAGE:42880 3', mRNA sequence. |
| 351 | 36580_at | 0.027139 Source: Homo sapiens mRNA; cDNA DKFZp586M141 (from clone DKFZp586M141). |
| 352 | 40387_at | 0.027139 similar to mouse vzg-1 |
| 353 | 38657_s_at | 0.027139 clathrin light-chain a |
| 354 | 33110_at | 0.027139 Source: qy22a10.x1 NCI_CGAP_Brn23 Homo sapiens cDNA clone IMAGE:2012730 3' similar to gb:X71136 SOX-10 PROTEIN (HUMAN);, mRNA sequence. |
| 355 | 36948_at | 0.027261 Source: Homo sapiens mRNA full length insert cDNA clone EUROIMAGE 45620. |
| 356 | 34354_at | 0.027592 putative |

## TABLE 7

## Gene Expression Changes at 4 Weeks in the Rostral Hypothalamus

| | | |
|---|---|---|
| 357 | 40779_at | 0.027592 Smg GDS-associated protein having arm repeats and phosphorylated by Src tyrosine kinase |
| 358 | 39072_at | 0.027634 Source: Human MXI1 mRNA, complete cds. |
| 359 | 1179_at | 0.027634 |
| 360 | 35768_at | 0.027634 Source: Homo sapiens mRNA for KIAA0661 protein, complete cds. |
| 361 | 37373_at | 0.027634 similar to uridine diphosphoglucose pyrophosphorylase in human liver, Swiss-Prot Accession Number Q07131; the 5'UTR and 3'UTR of this clone are completely different from those of the liver form |
| 362 | 41338_at | 0.027634 Source: wx39f10.x1 NCI_CGAP_Pit1 Homo sapiens cDNA clone IMAGE:2546059 3', mRNA sequence. |
| 363 | 35837_at | 0.027634 Source: Homo sapiens mRNA for scrapie responsive protein 1. |
| 364 | 1675_at | 0.027634 ras p21 GTP-ase-activating protein (GAP) |
| 365 | 37395_at | 0.027634 Source: Homo sapiens mRNA for vacuolar ATPase, complete cds. |
| 366 | 41759_at | 0.027634 Source: H. sapiens mRNA for RNA polymerase II elongation factor-like protein. |
| 367 | 36046_at | 0.027634 similarity to SPAC2F3.16 |
| 368 | 35468_at | 0.027854 Source: Homo sapiens mRNA; cDNA DKFZp586B2023 (from clone DKFZp586B2023). |
| 369 | 35403_at | 0.028018 Source: Homo sapiens mRNA for KIAA1094 protein, complete cds. |
| 370 | 38676_at | 0.028018 Source: zl96e07.r1 Stratagene corneal stroma (#937222) Homo sapiens cDNA clone IMAGE:512484 5', mRNA sequence. |
| 371 | 1318_at | 0.028018 Source: H. sapiens RbAp48 mRNA encoding retinoblastoma binding protein. |
| 372 | 33170_at | 0.028018 Source: Homo sapiens mRNA for KIAA0962 protein, partial cds. |
| 373 | 38190_r_at | 0.028018 Source: Homo sapiens mRNA for KIAA0645 protein, complete cds. |
| 374 | 35218_at | 0.028018 apoptosis-related protein |
| 375 | 41528_at | 0.02862 Source: zd62h08.s1 Soares_fetal_heart_NbHH19W Homo sapiens cDNA clone IMAGE:345279 3', mRNA sequence. |
| 376 | 37666_at | 0.02862 Source: Human mRNA for proteasome subunit X, complete cds. |
| 377 | 37229_at | 0.02862 similar to FRAP, Mec1p, Tor1p, Tor2p, and ATM |
| 378 | 38943_at | 0.028882 putative |
| 379 | 1074_at | 0.028882 Source: Homo sapiens GTP-binding protein (RAB1) mRNA, complete cds. |
| 380 | 1942_s_at | 0.028882 Cyclin-dependent kinase 4 |
| 381 | 411_i_at | 0.029002 Source: Human 1-8D gene from interferon-inducible gene family. |
| 382 | 32669_at | 0.029424 Source: Homo sapiens mRNA for KIAA0671 protein, complete cds. |
| 383 | 37543_at | 0.029842 this sequence overlaps D13631, it covers 954..4359 of this sequence. |
| 384 | 34845_at | 0.029842 remainder of gene in clone 549K18 (AL023654) |
| 385 | 38817_at | 0.029842 Source: Homo sapiens sperm acrosomal protein mRNA, complete cds. |
| 386 | 41825_at | 0.029966 Source: 34c6 Human retina cDNA randomly primed sublibrary Homo sapiens cDNA, mRNA sequence. |
| 387 | 41850_s_at | 0.030352 isolated in a two hybrid screen to identify cellular proteins that interact with hepatitis delta antigen; similar to hepatitis delta antigen, and has two regions predicted to form coiled-coil protein interaction domains |
| 388 | 40480_s_at | 0.0309 c-syn protooncogene; belongs to the protein-tyrosine kinase family of retroviral oncogenes |
| 389 | 1463_at | 0.0309 Source: Human protein tyrosine phosphatase (PTP-PEST) mRNA, complete cds. |
| 390 | AFFX-HUMISGF3A/M97935_MB_at | 0.0309 Source: Homo sapiens transcription factor ISGF-3 mRNA, complete cds. |
| 391 | 37774_at | 0.0309 Source: wj88e02.x1 NCI_CGAP_Lym12 Homo sapiens cDNA clone IMAGE:2409914 3' similar to SW:GBB5_HUMAN O14775 GUANINE NUCLEOTIDE-BINDING PROTEIN BETA SUBUNIT 5 ;, mRNA sequence. |
| 392 | 38832_r_at | 0.0309 M11319; Homo sapiens chromosome 7q22 sequence, complete sequence. |
| 393 | 33722_at | 0.031224 Source: Homo sapiens mRNA for KIAA0548 protein, partial cds. |

## TABLE 7

## Gene Expression Changes at 4 Weeks in the Rostral Hypothalamus

| | | |
|---|---|---|
| 394 | 36107_at | 0.031626 Source: ak04e09.s1 Soares_parathyroid_tumor_NbHPA Homo sapiens cDNA clone IMAGE:1405000 3' similar to gb:M37104 ATP SYNTHASE COUPLING FACTOR 6, MITOCHONDRIAL PRECURSOR (HUMAN);, mRNA sequence. |
| 395 | 39736_at | 0.031702 GTP-binding protein G25K |
| 396 | 38069_at | 0.032016 Source: H. sapiens mRNA for CLC-7 chloride channel protein. |
| 397 | 556_s_at | 0.032345 Source: Human glutathione transferase class mu number 4 (GSTM4) gene, complete cds. |
| 398 | 38336_at | 0.032745 hj06791 cDNA clone for KIAA1013 has a 4-bp deletion at position between 1855 and1860 of the sequence of KIAA1013. |
| 399 | 33913_at | 0.032745 HLA-B-associated transcript 2 (BAT2) |
| 400 | 33358_at | 0.03283 Source: 56b8 Human retina cDNA randomly primed sublibrary Homo sapiens cDNA, mRNA sequence. |
| 401 | 767_at | 0.032913 Source: Human Chromosome 16 BAC clone CIT987SK-A-815A9, complete sequence. |
| 402 | 35163_at | 0.033005 Source: Homo sapiens mRNA for KIAA1041 protein, complete cds. |
| 403 | 1278_at | 0.033089 |
| 404 | 1695_at | 0.033089 Source: Homo sapiens mRNA for ubiquitin-like protein, complete cds. |
| 405 | 34648_at | 0.033089 Source: H. sapiens mRNA for SSR alpha subunit. |
| 406 | 40211_at | 0.033171 Source: Human gene for heterogeneous nuclear ribonucleoprotein (hnRNP) core protein A1. |
| 407 | 34085_at | 0.033329 Source: H. sapiens gene for ribosomal protein L38. |
| 408 | 1635_at | 0.033329 ABL is the cellular homolog proto-oncogene of Abelson's murine leukemia virus and is associated with the t9:22 chromosomal translocation with the BCR gene in chronic myelogenous and acute lymphoblastic leukemia; alternative splicing using exon 1a |
| 409 | 37675_at | 0.033565 Source: H. sapiens mRNA for mitochondrial phosphate carrier protein. |
| 410 | 38110_at | 0.033587 mda-9; pbp-1 |
| 411 | 40771_at | 0.033587 match: proteins: Sw:P26038 Tr:O35763 Sw:P26041 Sw:P26042 Sw:P26044 Sw:P35241 Sw:P26043 Sw:P15311 Sw:P31976 Sw:P26040 Tr:Q26520 Tr:Q24788 Tr:Q24796 Tr:Q94815 |
| 412 | 869_at | 0.033639 general transcription factor IIA, 2 (12kD subunit) |
| 413 | 41203_at | 0.033639 putative |
| 414 | 40426_at | 0.033639 Source: H. sapiens mRNA for BCL7B protein. |
| 415 | 36032_at | 0.033639 partially supported by FGENES and GENSCAN |
| 416 | 39376_at | 0.033662 Source: Homo sapiens mRNA for KIAA0630 protein, partial cds. |
| 417 | 39380_at | 0.033775 Source: Homo sapiens mRNA for KIAA0697 protein, partial cds. |
| 418 | 40091_at | 0.033775 6 C2H2 zinc finger zinger repeats from 520 to 691 in protein sequence |
| 419 | 33866_at | 0.03399 tropomyosin (AA 1-248) |
| 420 | 34809_at | 0.03399 Source: yq87g03.r1 Soares fetal liver spleen 1NFLS Homo sapiens cDNA clone IMAGE:202804 5', mRNA sequence. |
| 421 | 39778_at | 0.03402 Source: Human N-acetylglucosaminyltransferase I (GlcNAc-TI) mRNA, complete cds. |
| 422 | 36121_at | 0.034483 Source: Homo sapiens mRNA for KIAA1065 protein, complete cds. |
| 423 | 41116_at | 0.034861 Source: wc43d09.x1 NCI_CGAP_Pr28 Homo sapiens cDNA clone IMAGE:2321393 3' similar to SW:YD84_SCHPO Q10409 HYPOTHETICAL 32.6 KD PROTEIN C1F3.04C IN CHROMOSOME I. ;, mRNA sequence. |
| 424 | 34781_at | 0.034861 Source: Human WS-3 mRNA, complete cds. |
| 425 | 34812_at | 0.034907 Source: 22f11 Human retina cDNA randomly primed sublibrary Homo sapiens cDNA, mRNA sequence. |
| 426 | 32276_at | 0.035041 rpL32 (aa 1-135) |
| 427 | 36636_at | 0.035115 Source: Human ornithine aminotransferase mRNA, complete cds. |
| 428 | 37674_at | 0.035221 5-aminolevulinate synthase precursor |
| 429 | 41814_at | 0.035459 alpha-L-fucosidase precursor (EC 3.2.1.5) |
| 430 | 37907_at | 0.035531 CpG island protein |
| 431 | 35195_at | 0.03601 Source: H. sapiens mRNA for phosphate cyclase. |

## TABLE 7
## Gene Expression Changes at 4 Weeks in the Rostral Hypothalamus

| | | |
|---|---|---|
| 432 | 35720_at | 0.036032 Source: Homo sapiens mRNA for KIAA0893 protein, complete cds. |
| 433 | 34600_s_at | 0.036032 Source: Human tub homolog mRNA, complete cds. |
| 434 | 32526_at | 0.036255 hypothetical protein FLJ14529 |
| 435 | 33295_at | 0.036255 Source: H. sapiens DARC gene. |
| 436 | 41404_at | 0.036255 Source: Homo sapiens mRNA for Ribosomal protein kinase B (RSK-B). |
| 437 | 41170_at | 0.036255 Source: Homo sapiens mRNA for KIAA0663 protein, complete cds. |
| 438 | 34774_at | 0.036255 Source: Human palmitoyl protein thioesterase mRNA, complete cds. |
| 439 | 35670_at | 0.036255 Source: Human Na+,K+ -ATPase catalytic subunit alpha-III isoform gene, exon 23, clone lambda-NK-alpha-R3-2. |
| 440 | 33835_at | 0.036255 Source: Homo sapiens mRNA for KIAA0721 protein, partial cds. |
| 441 | 35756_at | 0.036255 Source: Homo sapiens RGS-GAIP interacting protein GIPC mRNA, complete cds. |
| 442 | 38679_g_at | 0.036255 Source: zg79b05.s1 Soares_fetal_heart_NbHH19W Homo sapiens cDNA clone IMAGE:399537 3' similar to gb:M65125_ma1 U1 AND U2 SMALL NUCLEAR RIBONUCLEOPROTEIN E (HUMAN);, mRNA sequence. |
| 443 | 1081_at | 0.036261 ornithine decarboxylase |
| 444 | 41559_at | 0.036261 Source: zw24f03.r1 Soares ovary tumor NbHOT Homo sapiens cDNA clone IMAGE:770237 5', mRNA sequence. |
| 445 | 40587_s_at | 0.03647 Source: Homo sapiens p18 protein mRNA, complete cds. |
| 446 | 1884_s_at | 0.03647 cyclin |
| 447 | 37348_s_at | 0.036478 Source: ak01g01.s1 Soares_parathyroid_tumor_NbHPA Homo sapiens cDNA clone IMAGE:1404720 3' similar to SW:TRI7_HUMAN Q15651 THYROID RECEPTOR INTERACTING PROTEIN 7 ;, mRNA sequence. |
| 448 | 37759_at | 0.036478 Source: Human lysosomal-associated multitransmembrane protein (LAPTm5) mRNA, complete cds. |
| 449 | 40182_s_at | 0.036776 Source: Homo sapiens clone 24658 mRNA sequence. |
| 450 | 32543_at | 0.036921 Source: Human autoantigen calreticulin mRNA, complete cds. |
| 451 | 32750_r_at | 0.036934 actin-binding protein |
| 452 | 117_at | 0.036934 heat-shock protein HSP70B |
| 453 | 32548_at | 0.036934 Source: Human (p23) mRNA, complete cds. |
| 454 | 41594_at | 0.036934 Source: Human protein-tyrosine kinase (JAK1) mRNA, complete cds. |
| 455 | 37732_at | 0.036934 Source: Homo sapiens mRNA; cDNA DKFZp564E1922 (from clone DKFZp564E1922). |
| 456 | 41128_at | 0.03697 Source: Homo sapiens clone 24606 mRNA sequence. |
| 457 | 35903_at | 0.037598 Source: Human oligodendrocyte-myelin glycoprotein (OMGP) mRNA, complete cds. |
| 458 | 33809_at | 0.037604 GTP-binding regulatory protein Gi alpha-1 chain |
| 459 | 33466_at | 0.037604 Source: Homo sapiens clone 23860 mRNA sequence. |
| 460 | 39897_at | 0.037937 Source: yy39g07.s1 Soares melanocyte 2NbHM Homo sapiens cDNA clone IMAGE:273660 3', mRNA sequence. |
| 461 | 34950_at | 0.037937 Source: Homo sapiens mRNA for KIAA0760 protein, partial cds. |
| 462 | 41530_at | 0.037937 Source: Human mRNA for mitochondrial 3-oxoacyl-CoA thiolase, complete cds |
| 463 | 34753_at | 0.037937 Source: H. sapiens mRNA for novel gene in Xq28 region. |
| 464 | 36160_s_at | 0.037937 Source: Human protein tyrosine phosphatase receptor pi (PTPRP) mRNA, complete cds. |
| 465 | 34593_g_at | 0.037937 ribosomal protein S17 |
| 466 | 34752_at | 0.038065 Source: Homo sapiens mRNA; cDNA DKFZp586G2222 (from clone DKFZp586G2222). |
| 467 | 34213_at | 0.038065 Source: Homo sapiens mRNA for KIAA0869 protein, partial cds. |
| 468 | 33893_r_at | 0.038065 Source: Homo sapiens mRNA for KIAA0470 protein, complete cds. |
| 469 | 34392_s_at | 0.038065 Rab1, splice variant |
| 470 | 38045_at | 0.038065 a novel member of the Armadillo family interacting with presenilin 1 (PS1); presenilin associated protein |

## TABLE 7

## Gene Expression Changes at 4 Weeks in the Rostral Hypothalamus

| | | |
|---|---|---|
| 471 | 38062_at | 0.038065 Similar to a C.elegans guanine nucleotide releasing factor homolog (S4 2368) |
| 472 | 38573_at | 0.038065 zinc finger protein |
| 473 | 32119_at | 0.038065 Source: Homo sapiens mRNA; cDNA DKFZp586B211 (from clone DKFZp586B211). |
| 474 | 31955_at | 0.038065 Source: H. sapiens fau mRNA. |
| 475 | 31899_at | 0.038065 Source: Human mRNA for KIAA0103 gene, complete cds. |
| 476 | 38991_at | 0.038065 Source: HSU55980 Human brain ARSanders Homo sapiens cDNA clone 25453 3', mRNA sequence. |
| 477 | 39809_at | 0.038065 HBP1 |
| 478 | 41375_at | 0.038065 Source: Homo sapiens mRNA for G7b protein (G7b gene, located in the class III region of the major histocompatibility complex. |
| 479 | 1356_at | 0.038065 Source: Human ionizing radiation resistance conferring protein mRNA, complete cds. |
| 480 | 39009_at | 0.038065 Source: yy66d08.r1 Soares_multiple_sclerosis_2NbHMSP Homo sapiens cDNA clone IMAGE:278511 5', mRNA sequence. |
| 481 | 39327_at | 0.038065 similar to D.melanogaster peroxidasin(U11052) |
| 482 | 41449_at | 0.038065 Source: Homo sapiens mRNA for epsilon-sarcoglycan. |
| 483 | 40775_at | 0.038065 5' end of gene beyond this clone; match: protein Q61500 |
| 484 | 36511_at | 0.038065 Source: Homo sapiens mRNA for KIAA0851 protein, complete cds. |
| 485 | 35833_at | 0.038065 Source: Homo sapiens mRNA; cDNA DKFZp434O071 (from clone DKFZp434O071). |
| 486 | 35845_at | 0.038065 Source: Homo sapiens mRNA for Sec24 protein (Sec24B isoform). |
| 487 | 36271_at | 0.038065 Source: Homo sapiens mRNA for KIAA1024 protein, partial cds. |
| 488 | 41715_at | 0.038065 Source: H. sapiens mRNA for phosphoinositide 3-kinase. |
| 489 | 41665_at | 0.038065 Source: Homo sapiens mRNA for KIAA0824 protein, partial cds. |
| 490 | 35342_at | 0.038065 Source: Homo sapiens clone 24416 mRNA sequence. |
| 491 | 41799_at | 0.038065 Source: 48h8 Human retina cDNA randomly primed sublibrary Homo sapiens cDNA, mRNA sequence. |
| 492 | 36603_at | 0.038065 similar to Yeast translation activator GCN1 (P1:A48126) |
| 493 | 39766_r_at | 0.038229 Source: tr08h04.x1 NCI_CGAP_Ov23 Homo sapiens cDNA clone IMAGE:2217751 3' similar to SW:RPCX_HUMAN P53803 DNA-DIRECTED RNA POLYMERASES I, II, AND III 7.0 KD POLYPEPTIDE ;, mRNA sequence. |
| 494 | 38100_at | 0.038407 Source: Homo sapiens mRNA for translocation protein-1, complete cds. |
| 495 | 39441_at | 0.038506 Source: Homo sapiens mRNA for lanthionine synthetase C-like protein 1 (LANCL1 gene). |
| 496 | 38398_at | 0.038994 Source: Human mRNA for KIAA0358 gene, complete cds. |
| 497 | 33108_i_at | 0.039132 putative |
| 498 | 35102_at | 0.039332 Source: Homo sapiens zinc finger protein mRNA, 3' end. |
| 499 | 2065_s_at | 0.039432 Source: Human Bax alpha mRNA, complete cds. |
| 500 | 34814_at | 0.039631 Source: DKFZp434D0717_s1 434 (synonym: htes3) Homo sapiens cDNA clone DKFZp434D0717 3', mRNA sequence. |
| 501 | 41436_at | 0.039781 Source: Homo sapiens mRNA for ZNF198 protein. |
| 502 | 33451_s_at | 0.039781 Source: DU3.2-7.G09 DU-145 Homo sapiens cDNA 3', mRNA sequence. |
| 503 | 37489_s_at | 0.039781 Source: Human anion exchanger 3 brain isoform (bAE3) mRNA, complete cds. |
| 504 | 36650_at | 0.040102 Source: Human mRNA for KIAK0002 gene, complete cds. |
| 505 | 34849_at | 0.040102 Source: H. sapiens mRNA for seryl-tRNA synthetase. |
| 506 | 743_at | 0.040122 putative |
| 507 | 1072_g_at | 0.040122 Source: Human transcription factor GATA-2 (GATA-2) mRNA, complete cds. |
| 508 | 1660_at | 0.040122 Source: Human epidermoid carcinoma mRNA for ubiquitin-conjugating enzyme E2 similar to Drosophila bendless gene product, complete cds. |
| 509 | 428_s_at | 0.040122 Source: Human messenger RNA fragment for the beta-2 microglobulin. |
| 510 | 837_s_at | 0.040122 Source: Human breast cancer cytosolic NADP(+)-dependent malic enzyme mRNA, partial cds. |

## TABLE 7

## Gene Expression Changes at 4 Weeks in the Rostral Hypothalamus

| | | |
|---|---|---|
| 511 | 38652_at | 0.040388 Source: Homo sapiens clone 24742 mRNA sequence. |
| 512 | 33301_g_at | 0.040401 |
| 513 | 35307_at | 0.040403 Source: Homo sapiens mRNA for GDP dissociation inhibitor beta. |
| 514 | 40537_at | 0.040659 Source: Homo sapiens mRNA for KIAA0741 protein, complete cds. |
| 515 | 40018_at | 0.040659 Source: Homo sapiens KIAA0410 mRNA, complete cds. |
| 516 | 38993_r_at | 0.040683 Source: 32a12 Human retina cDNA randomly primed sublibrary Homo sapiens cDNA, mRNA sequence. |
| 517 | 33369_at | 0.040683 Source: P9-C4.T3.P9.D4 conorm Homo sapiens cDNA 3', mRNA sequence. |
| 518 | 38065_at | 0.040683 Source: H. sapiens HMG-2 mRNA. |
| 519 | 630_at | 0.040702 Source: Homo sapiens deoxycytidylate deaminase gene, complete cds. |
| 520 | 39026_r_at | 0.040775 Source: Homo sapiens clone 23887 mRNA sequence. |
| 521 | 37298_at | 0.040775 Source: Homo sapiens MM46 mRNA, complete cds. |
| 522 | 1566_at | 0.040775 145 kda neural cell adhesion molecule; This sequence comes from Fig. 4 |
| 523 | 1226_at | 0.041055 transmembrane metalloproteinase/disintegrin; adamalysin; TACEA |
| 524 | 36165_at | 0.041055 Source: zc48b04.r1 Soares_senescent_fibroblasts_NbHSF Homo sapiens cDNA clone IMAGE:325519 5' similar to gb:X13238 CYTOCHROME C OXIDASE POLYPEPTIDE VIC PRECURSOR (HUMAN);, mRNA sequence. |
| 525 | 32510_at | 0.041215 2-carboxybenzaldehyde reductase; member of aldo-keto reductase AKR7 family |
| 526 | 39791_at | 0.041257 Source: Homo sapiens calcium-ATPase (HK1) mRNA, complete cds. |
| 527 | 31809_at | 0.041257 Source: H. sapiens contactin mRNA,. |
| 528 | 36100_at | 0.041257 Source: Homo sapiens vascular endothelial growth factor mRNA, complete cds. |
| 529 | 33924_at | 0.041338 Source: Homo sapiens mRNA for KIAA1091 protein, partial cds. |
| 530 | 40846_g_at | 0.041338 Source: Human nuclear factor NF90 mRNA, complete cds. |
| 531 | 38664_at | 0.041338 Source: Homo sapiens BCNT mRNA, complete cds. |
| 532 | 41283_at | 0.041494 Source: Homo sapiens clone 23930 mRNA sequence. |
| 533 | 38042_at | 0.041843 G6PD (AA 1-515) |
| 534 | 1627_at | 0.041843 |
| 535 | 35152_at | 0.041927 Source: Homo sapiens mRNA encoding RAMP3. |
| 536 | 33864_at | 0.041927 binds directly to adenovirus type 5 E1A protein |
| 537 | 32254_at | 0.041979 Source: Homo sapiens mRNA; cDNA DKFZp586L1323 (from clone DKFZp586L1323). |
| 538 | 40435_at | 0.041983 ADP.ATP translocase |
| 539 | 37616_at | 0.041993 Source: H. sapiens AUH mRNA. |
| 540 | 1213_at | 0.042177 similar to human serine kinase SRPK1, encoded by GenBank Accession Number U09564; specific for serine/arginine-rich splicing factors |
| 541 | 818_s_at | 0.042207 XH2; XNP; alternatively spliced product 1; contains all exons; translation starts in exon 9; ATRX gene deposited in GenBank Accession Numbers U72900-U72935 |
| 542 | 37181_at | 0.042334 Source: H. sapiens Mpv17 mRNA. |
| 543 | 35776_at | 0.042686 Source: Homo sapiens intersectin short form mRNA, complete cds. |
| 544 | 31820_at | 0.042778 haematopoietic lineage cell protein (AA 1-486) |
| 545 | 41344_s_at | 0.042778 Source: H. sapiens Pur (pur-alpha) mRNA, complete cds. |
| 546 | 1468_at | 0.042778 TNF type I receptor associated protein |
| 547 | 38306_at | 0.043327 Source: zu44b03.r1 Soares ovary tumor NbHOT Homo sapiens cDNA clone IMAGE:740813 5', mRNA sequence. |
| 548 | 34326_at | 0.043502 Source: H. sapiens mRNA for beta-COP. |
| 549 | 39442_at | 0.043534 unc-50 related protein homolog |
| 550 | 35619_at | 0.043649 Source: Homo sapiens mRNA for KIAA0634 protein, partial cds. |
| 551 | 1953_at | 0.043649 Vascular endothelial growth factor |

## TABLE 7

## Gene Expression Changes at 4 Weeks in the Rostral Hypothalamus

| | | |
|---|---|---|
| 552 | 37580_at | 0.043649 predicted; contains SH3 domain; member of the EEN gene family |
| 553 | 33351_at | 0.044111 similar to SUI1 |
| 554 | 33752_at | 0.044234 Source: Homo sapiens mRNA for KIAA0850 protein, complete cds. |
| 555 | 41542_at | 0.044234 Source: Homo sapiens zinc finger protein 216 splice variant 1 (ZNF216) mRNA, complete cds. |
| 556 | 32853_at | 0.044234 Source: Homo sapiens mRNA for KIAA0719 protein, complete cds. |
| 557 | 34876_at | 0.044268 duck gp180 homolog |
| 558 | 35722_at | 0.044288 similarity to S.cerevisiae Nmd2p |
| 559 | 40636_at | 0.044411 Source: wf49b01.x1 Soares_NFL_T_GBC_S1 Homo sapiens cDNA clone IMAGE:2358889 3' similar to TR:O75955 O75955 FLOTILLIN-1. ;, mRNA sequence. |
| 560 | 33443_at | 0.044411 match: proteins P38533 Q03933 P38530 P41154 Q00613 P38529 P38531 Q63717 P38532 Q99472 |
| 561 | 555_at | 0.044411 small GTP binding protein, homologous to Saccharomyces cerevisiae SEC4, Swiss-Prot Accession Number P07560 |
| 562 | 38120_at | 0.044659 autosomal dominant polycystic kidney disease type II |
| 563 | 40263_at | 0.045291 Source: Homo sapiens clone lambda MEN1 region unknown protein mRNA, complete cds. |
| 564 | 31797_at | 0.046069 Source: Human DNA sequence from clone 73H22 on chromosome 6q23, complete sequence. |
| 565 | 41194_at | 0.046069 Source: PT1.3_04_C04.r tumor1 Homo sapiens cDNA 5', mRNA sequence. |
| 566 | 37532_at | 0.0468 Source: Human medium-chain acyl-CoA dehydrogenase (MCAD) gene, exon 12. |
| 567 | 883_s_at | 0.0468 Source: Human h-pim-1 protein (h-pim-1) mRNA, complete cds. |
| 568 | AFFX-HUMRGE/M10098_5_at | 0.0468 Source: Human 18S rRNA gene, complete. |
| 569 | 39033_at | 0.047068 Source: HSZ78368 Human fetal brain S. Meier-Ewert Homo sapiens cDNA clone 3.142 (CEPH), mRNA sequence. |
| 570 | 33249_at | 0.047068 mineralocorticoid receptor |
| 571 | 36480_at | 0.047068 alpha subunit |
| 572 | 36563_at | 0.047068 Source: Homo sapiens clone 23582 mRNA sequence. |
| 573 | 35746_r_at | 0.047095 Source: H. sapiens hnRNP-E2 mRNA. |
| 574 | 38743_f_at | 0.047214 Source: Human mRNA fragment for activated c-raf-1 (exons 8-17). |
| 575 | 41110_at | 0.047214 Source: H. sapiens mRNA for for vasopressin activated calcium mobilizing receptor-like protein. |
| 576 | 35187_at | 0.048079 Source: Homo sapiens mRNA; cDNA DKFZp586K1123 (from clone DKFZp586K1123). |
| 577 | 41296_s_at | 0.048079 Source: 37c5 Human retina cDNA randomly primed sublibrary Homo sapiens cDNA, mRNA sequence. |
| 578 | 781_at | 0.048079 subunit of modification enzyme; beta subunit |
| 579 | 32228_at | 0.048079 Source: Homo sapiens mRNA for KIAA0899 protein, partial cds. |
| 580 | 35090_g_at | 0.048079 Source: Homo sapiens mRNA for NTAK, complete cds. |
| 581 | 457_s_at | 0.048079 conjugated post-translationally to RanGAP1; ubiquitin-related protein; similar to UBL1 encoded by GenBank Accession Number U38784, PIC1 encoded by GenBank Accession Number U61397 and GMP1 encoded by GenBank Accession Number U72722 |
| 582 | 37333_at | 0.048079 Source: H. sapiens mRNA for DNA (cytosin-5)-methyltransferase. |
| 583 | 35683_at | 0.048079 Source: Homo sapiens mRNA for KIAA0852 protein, complete cds. |
| 584 | 1253_at | 0.048079 Source: Human protein kinase mRNA, complete cds. |
| 585 | 31807_at | 0.04809 Source: U69190 Soares infant brain 1NIB Homo sapiens cDNA clone 27655, mRNA sequence. |
| 586 | 430_at | 0.048334 PNP |
| 587 | 1280_i_at | 0.048396 |
| 588 | 37274_at | 0.048396 biotin-amide amidohydrolase |
| 589 | 34840_at | 0.048884 Source: we38g03.x1 NCI_CGAP_Lu24 Homo sapiens cDNA clone IMAGE:2343412 3', mRNA sequence. |
| 590 | 40308_at | 0.049324 Source: wh51h03.x1 NCI_CGAP_Kid11 Homo sapiens cDNA clone IMAGE:2384309 3', mRNA sequence. |

## TABLE 7
## Gene Expression Changes at 4 Weeks in the Rostral Hypothalamus

| | | |
|---|---|---|
| 591 | 41573_at | 0.049324 Source: H. sapiens SPR-2 mRNA for GT box binding protein. |
| 592 | 34739_at | 0.049324 Source: 18c3 Human retina cDNA randomly primed sublibrary Homo sapiens cDNA, mRNA sequence. |
| 593 | 40517_at | 0.049343 Source: Human mRNA for KIAA0372 gene, complete cds. |
| 594 | 35983_at | 0.049343 Hypothetical human protein (partial CDS); CDS constructed from combination of BLASTX, EST matches and Xgrail predictions. N-terminus of protein likely encoded in flanking cosmid R29942. Predicted protein exhibits weak similarity to hypothetical protein PID\|e1226191 (AL021106) from Drosophila melanogaster |
| 595 | 37895_at | 0.049343 Source: Homo sapiens mRNA for CMP-sialic acid transporter, complete cds. |
| 596 | 33877_s_at | 0.049343 Source: Homo sapiens mRNA for KIAA1067 protein, partial cds. |
| 597 | 34368_at | 0.049343 similar to yeast RPD3, encoded by GenBank Accession Number X78454 |
| 598 | 41266_at | 0.049343 Source: Human mRNA for integrin alpha 6. |
| 599 | 32589_at | 0.049516 Source: Human chromatin assembly factor-I p150 subunit mRNA, complete cds. |
| 600 | 36907_at | 0.049516 Source: Homo sapiens mevalonate kinase mRNA, complete cds. |
| 601 | 35703_at | 0.049564 PDGF-A (AA 1 - 196) |
| 602 | 31932_f_at | 0.04966 Source: Human basic transcription factor 3a (BTF3a) gene, complete cds |

[00106] Serum levels were determined for IGFB2 and found to be decreased, in agreement with the decreased transcript levels in several organs, including the liver.

## TABLE 8
## Serum Determination of Insulin-Like Growth Factor Binding Protein 2

| Treatment | conc (ng/mL) | mean (ng/mL) |
|---|---|---|
| control | 1467 | 1583 |
| | 1742 | |
| | 922 | |
| | 2201 | |
| FGF23CTP 100 µg/day | 1239.8 | 893 |
| | 987.6 | |
| | 596.7 | |
| | 749.5 | |

[00107] *Analysis*. Angiogenesis had previously been inhibited by FGF23CTP in a hyperoxia-induced angioproliferative retinopathy model (Aiello FP *et al., Proc. Natl. Acad. Sci. USA*, 92: 10457-61 (1995), Ozaki H *et al., Am. J. Pathol.* 156: 697-707 (2000)) in C57/B6 mice. The proliferative component of the retinal angiopathy induced through a relative ischemia by transition of postnatal mice from hyperoxic to normoxic conditions was significantly inhibited (p=0.018) by the intravitreal injection of the FGF23CTP (GPA006) peptide.

[00108] In the rostral hypothalamus, besides its effects on angiogenesis and cycling genes, FGF23CTP affects several molecules that have been described to play a role in the pathogenesis of malignant proliferation of glial cells and precursors (malignant brain tumors): epithelial growth factor (EGF; Hoi Sang U *et al., J. Neurosurg.* 82: 841-6 (1995), Wu CJ *et*

*al.*, *Oncogene* 19: 3999-4010 (2000)), Bax (Streffer JR *et al.*, *J. Neurooncol.* 56: 43-9 (2002); Martin *et al.*, (2001)), connexin 43 (Huang R *et al.*, *Cancer Res.* 62: 2806-12 (2002), Soroceanu L *et al.*, *Glia* 33: 107-17 (2001)), PKR (Shir A & Levitzki A, *Nature Biotechnology* 20: 895-900 (2002)), NF1 (Cichowski K & Jacks T, *Cell* 104: 593-604 (2001), Gutmann DH *et al.*, *Hum. Mol. Genet.* 10: 3009-16 (2001)).

EXAMPLE IV

INTEGRATED INVESTIGATIVE PHARMACOLOGY THROUGH *IN VIVO* GENE EXPRESSION PROFILING IN NON-HUMAN PRIMATES

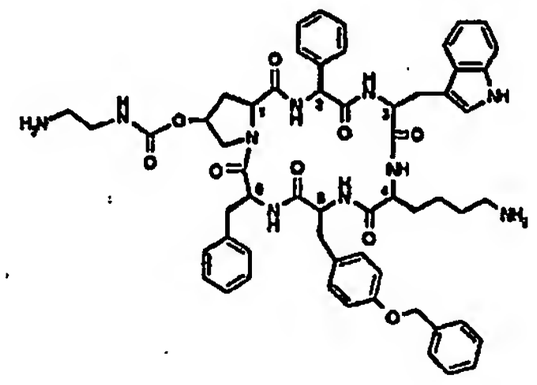
[00109] The discovery method of the invention has been validated through a "blind" monkey trial using three peptides of well known pharmacological activity: (1) the somatostatin analogue SOM230, (2) gonadotrophin releasing hormone (GnRH), (3) and leukemia inhibitory factor (LIF). In each case, a "blind" test with 3 "unknown" polypeptides was performed. The results demonstrated the capacity of the gene expression analyst teams to identify, within four months, the pharmacological activities, most of the therapeutic indications and side effects, and even the identity of the proteins. These first results demonstrate that the discovery method of the invention usefully provide the art with advantage in the understanding of drug pharmacological mechanisms and the potential side effects, the selection of biomarkers and potential new indications.

[00110] For the verification of the selected human proteins or peptides in cynomolgus monkeys, one control and four treated groups (*i.e.*, each of three peptides and placebo) of two males and two females are treated for two weeks by daily administration of the proteins dissolved in autologous serum (*e.g.*, in each animal's own serum) through the subcutaneous route. The administration of the peptides was blinded. The amount of peptide administered was 100 μg/animal /day (5-6 mg of peptide total).

[00111] Drug profiling in these non-human primates was analyzed using gene expression profiling of more than 100 organs on Affymetrix U95 chips (each containing 1/3 of the human genome). In addition, extensive biochemistry and clinical chemistry screening (> 60 parameters) and histopathology (*ca.* 60 organs) were performed.

[00112] Data mining procedures were then used. The data were probed to answer four questions: (1) Is the polypeptide worth further investigations? (2) What are the physiological pathways impacted? (3)What are the potential indications? (4) What is the likelihood of the identity of the polypeptide?

[00113] *Peptide 1*. Peptide 1 was SOM230. SOM230 (pasireotide) has a chemical structure cyclo[4-($NH_2$-$C_2H_4$-NH-CO-O)Pro-Phg-DTrp-Lys-Tyr(4-Bzl)-Phe] as follows:



(I)

[00114] Here, Phg means -HN-CH($C_6H_5$)-CO- and Bzl means benzyl. See, PCT patent application WO 02/10192. SOM230 is a somatostatin analogue with binding affinities for the five somatostatin receptors except somatostatin receptor 4 (SSTR4). SOM230 has been developed for several indications, including those disclosed above for other somatostatin analogues. See, Lewis I *et al., J. Med. Chem.* 46(12):2334-44 (June 5, 2003); Weckbecker G *et al., Endocrinology* 143(10): 4123-4130 (2002); Kneissel M *et al., Bone* 28:237-250 (2001); and Thomsen JS *et al., Bone* 25:561-569 (1999), the contents of which are incorporated herein by reference.

[00115] SOM230 was developed for the approved Sandostatin® indications, but as a more potent somatostatin analogue with a longer plasma half-life *in vivo*. Lewis I *et al., J Med Chem* 46(12): 2334-44 (June 5, 2003); Weckbecker G *et al., Endocrinology* 143(10): 4123-30 (October 2002). In contrast with other analogues, SOM230 binds to all somatostatin receptors except SSTR4. The binding affinity for the different somatostatin receptors was a basis for defining the scope of possible new clinical indications for SOM230. Bruns C *et al., Eur J Endocrinol* 143(Suppl 1): S3-7 (2000); Bruns C *et al., Eur J Endocrinol.* 146(5):707-16 (May 2002). In addition, other possible new indications were suggested due to the improved activity of SOM230 for growth hormone and IGF-1 regulation and its different inhibitory effects on insulin and glucagon secretions.

[00116] Following the blinded administration of peptide 1 (SOM230), the following results were obtained:

TABLE 9
Findings by the Data Mining Team

| Analysis | Results |
|---|---|
| Biochemistry/ Histopathology | Thymus and spleen atrophy, aldosterone-, glucagon-, vitamin D-, PTH+, RBC hemoglobin-, urinary calcium-, NAG+, TSH+ in male, |
| Pituitary | *IGFR1-*, GH1/2-, CSH(L)-, TSH-, CHRH2-, Prostaglandin D2 synthase++, Immunoglobulin-- |
| Adrenals | IGF2-, somatostatin-, insulin receptor+, urocortin+ |
| Pancreas | *Metallothionein 1A,1E++*, trypsin, chymotrypsin, carboxypeptidases, phospholipaseA2/1B ++, *18S--*, |
| Stomach | *Gastrin+*, hypocretin+, glutamate receptor+, GABAB-, IGFBP1+, |
| Thymus | MHC classII-, Ig-, FKBP12-, CD4-, defensin alpha1+, |
| Bone marrow | VIP-, peptide YY-, cyclin-, GAPDH- |
| Spleen | *IGF2-*, IGBP6-, insulin receptor-, urocortin+, Ig-, ribosomal protein --, Nk cells+ |
| Thyroid | NPY-, cyclins--, ATPase calcium channel+, somatostatin receptor 4-, |
| Liver | *IGF2-*, NMDA receptor+, glutamate dehydrogenase+, VIP, insulin-induced protein1, *STR5* |
| Kidney | Solute carrier protein 9++, urocortin+, IGF2-, arrestin beta 2-, THRα+, |

[00117] The underlined results in the TABLE above were identified for further investigation.

TABLE 10
Peptide 1: Conclusions of the *in vivo* Profiling by the Data Mining Team

| Function/Pathway | Potential Indications |
|---|---|
| Anti-growth activity | Cytostolic; cancer |
| GI tract/pancreatic function | Obesity, food intake regulation, diabetes, pancreatic function regulation |
| Immunosuppression | Suppression of specific immunity, maintenance of innate immunity |

[00118] Based upon these results, the Data Mining Team made predictions as to the identity of the administered Peptide 1.

TABLE 11

Predicted Identity of Peptide 1 (Somatostatin Analogue SOM230)

| Data miner | Prediction |
|---|---|
| 1 | Somatostatin, NPY, |
| 2 | IGF-1, Somatostatin, IGF-2 |
| 3 | Somatostatin, IGF-1, IGF-2 |
| 4 | Somatostatin, IGF-1, IGF-2 |
| 5 | Secretin, IGF-1, Renin |
| Summary Prediction | Somatostatin, IGF-1, IGF-2 |

[00119] Based upon these results, SOM230 was selected for further development as a somatostatin analogue to treat the somatostatin-approved neuroendocrine indications (*e.g.*, acromegaly, gastroenteropancreatic tumors) and also has potential for further indications with a pancreatic endocrine etiology such as diabetic angiopathy and morbid obesity associated with hyperinsulinemia. IGF-1 serum level was proposed as surrogate marker. Other potential indications for SOM230 are therefore inflammation (*e.g.*, psoriasis), pain and immunosuppression (*e.g.*, chronic rejection).

[00120] *Peptide 2*. Following the blinded administration of peptide 2 (gonadotrophin releasing hormone), the following results were obtained:

TABLE 12
Findings by the Data Mining Team

| Analysis | Results |
|---|---|
| Biochemistry | Slight increase in aldosterone |
| Kidney | Thiol protease inhibitor (aldosterone + in biochemistry), metabolism: +GAPDH, +STAT, +JAK, +TEGT |
| Pituitary Gland | *GH-*, *GNRH receptor-*, Somatostatin+, *FSH+*, *LH-*, TSH-, CSH(L)-, IGF/IGFBP-/+, tubulin+, |
| Ovary | *Nuclear receptors-*, *aromatase and estrogen synthase+*, activinA-, inhibin beta B+, |
| Testis | *CREM+* |
| Thyroid | Ocludin+, thyroid hormone binding protein+, THR alpha- |
| Adrenals | Follistatin+, IGF2+, catecholamines synthesis+, activin A-/inhibin+ |
| Hypothalamus | Transthyretin+, oxytocin |
| Bone marrow | Macrophages+, eosinophils+, activin A-, inhibin betaB+, |
| Thymus | Integrin alpha6+, VAP1+, lipid metabolism, selenium binding protein+, |
| Mammary gland | Inconclusive; Sampling heterogenous |
| Liver | Activin A-, inhibin betaB+, lipid metabolism+, glycolysis+, follistatin+, IDO1+, VEGF- |

[00121] The underlined results in the TABLE above were identified for further investigation.

TABLE 13
Peptide 2: Conclusions of the *in vivo* Profiling by the Data Mining Team

| Function/Pathway | Potential Indications |
|---|---|
| Ovarian and testicular functions | Fertility, stimulation of reproductive function, stimulation of steroidogenesis |

[00122] Based upon these results, the Data Mining Team made predictions as to the identity of the administered Peptide 2.

TABLE 14
Predicted Identity of Peptide 2 (GnRH)

| Data miner | Prediction |
|---|---|
| 1 | GnRH, FSH, HCG |
| 2 | FSH, IGF1, GnRH |
| 3 | FSH, Somatotropin, LH |
| 4 | GnRH, FSH, IGF1 |
| 5 | FSH, LHRH, IGF-1 |
| Summary Prediction | FSH, GnRH, LH-(RH) |

[00123] Based upon these results, the predicted indications for the administered Peptide 2 (gonadotropin releasing hormone) were be for gonadotrophin releasing hormone-related indications or for lutenizing hormone releasing hormone-related indications, such as antiproliferative disorders (cancer), ovarian and testicular functions (hypothalamic gonadotropc hypogonadism, fertility control and delayed or arrested puberty/ precocious puberty), and growth hormone deficiencies.

[00124] *Peptide 3*. Following the blinded administration of peptide 3 (leukemia inhibitory factor), the following results were obtained:

TABLE 15
Findings by the Data Mining Team

| Analysis | Results |
|---|---|
| Biochemistry/ Hematology | *Calcitonin*++++, osteocalcin+, PTH-, bone alkaline phosphatase-, IC terminal polypeptide-, T4+, TSH+, alpha globin1+, platelet count +, WBC- |
| Liver | C/EBP delta+, JunB+, NFIL3+, CREM, quiescin Q6+, BTG2+, TCR-, BCL6+, antileukoproteinase+, selectinP+, VCAM+, ephrin A1+, *Ostepontin*+, *calcitonin*+, TIMP-1, , plasminogen inhibitor+, IL1R1, cholesterol hydrolase+, LDL receptor, phospholipase AII type II + |
| Kidney | C/EBP delta+, JunB+, NFIL3+, CREM, immunoglobulin-, MCP1+, mannose receptor+, BCR+, VCAM+, *ephrinA2+*, osteopontin+, stanniocalcin+, *TIMP-1+*, urokinase+, ILR1+, LDL receptor, phospholipase AII type II + |
| Thyroid | C/EBP delta+, JunB+, NFIL3+, CREM, STAT3+, CXCR4+, myeloid leukemia sequence+, *Ig-*, ephrinA1+, osteopontin+, TIMP-1+, haptoglobin, fibronectin+, phospholipase A2 typeIIA+, PDGF+ |
| Skeletal muscle | JunB, haptoglobin, phospholipase A2 typeIIA+ |
| Spleen | JunB+, STAT inhibitor 3, calgranulinB+, CD5+, BCR+, ephrin A1 exostoses+, TIMP-1 +, fibronectin+, ILR1+, LDL receptor+ |
| Adrenals | C/EBP delta+, JunB+, NFIL3+, CREM, BCR+, TIMP-1+, urokinase+, IL1R1+, LDL receptor |
| Pituitary | C/EBP delta+, JunB+, NFIL3+, TCR-, BCL6+,, antileukoproeinase+, ephrin A1+, osteopontin+, chitinase 3-like1+, TIMP-1, plasminogen activator inhibitor, metallothionein+, haptoglobin+, IL1R1+ |

[00125] The underlined results were identified for further investigation.

TABLE 16
Peptide 3: Conclusions of the *in vivo* Profiling by the Data Mining Team

| Function/Pathway | Potential Indications |
|---|---|
| $Ca^{++}$ bone metabolism | Osteoarthritis, Osteoporosis |
| Inflammation | |
| Immunosuppression | Transplantation |
| Vascular differentiation growth factors | Angiogenesis |

[00126] Based upon these results, the Data Mining Team made predictions as to the identity of the administered Peptide 2.

TABLE 17
Predicted Identity of Peptide 3 (LIF)

| Data miner | Prediction |
|---|---|
| 1 | TGFβ2, IL-6, IFN gamma |
| 2 | TGFβ2, IL-6, Calcitonin |
| 3 | VIP |
| 4 | PDGF, GMCSF, Sertonin |
| 5 | LIF, IL3, TGFβ2 |
| 6 | Oncostatin M, TGFβ2, IL3 |
| Summary Prediction | IL-6, IL-3, TGFβ2, LIF/Onccostatin |

[00127] Based upon these results, the predicted actions for the administered Peptide 3 would be to increase platelets, myeloid cells and megakaryocytes; to increase acute phase proteins (such as C-Reactive Protein (CRP)and haptoglobin); to decrease lipogenicity (with therapeutic indications for treating obesity and cardiovascular risk); to decrease transaminase, albumin and lactate dehydrogenase (LDH); and to decrease alkaline phosphatase, bone sialoprotein (BSP) and osteocalcin (with therapeutic indications for treating osteoarthritis and osteoporosis).

[00128] In summary, the three questions that were posed to the data miners could be answered almost fully by the data miners. All three polypeptides were identified as being worth further investigation. All of the physiological pathways known to be impacted by these three administered polypeptides were discovered (*e.g.* bone fractures for LIF, gastrointestinal tract for SOM230). Eighty % of their known potential indications were discovered and some new ones were identified.

EXAMPLE V

SOM230-INDUCED GENE EXPRESSION PROFILING IN MONKEYS

[00129] *Introduction and summary.* Microarray gene expression assays were performed using tissues of monkeys treated with SOM230 at sub-therapeutic dose for 14 days. The assays were analyzed to identify the modes of actions of SOM230 with possible relationships to therapeutic applications. For a description of SOM230, see the EXAMPLE above.

[00130] All monkey tissues examined (thyroid, brown fat, pituitary, pancreas, liver, kidney, spleen) demonstrated changes in the genes regulated by the binding of the natural somatostatin 14 (SST-14) and somatostatin 28 (SST-28) to somatostatin receptors (SSTRs). The transcript profiles reflected the known somatostatin actions on the growth

hormone/insulin-like growth factor 1 (GH/IGF-1), glucagon/insulin axes and on cell proliferation. However, the compound affected significantly the transcript levels of other related genes like insulin-like growth factor 2 (IGF-2) in the pituitary and kidneys. This could be a candidate biological marker (biomarker) of drug efficacy provided that the change in protein biosynthesis would be reflected in an easily accessible tissue like the blood. Other known effects of somatostatin and agonists on growth factors, cells of the immune system and the cardio-vascular and renal functions were also reflected by the changes in the profiles of these classes of genes after SOM230.

[00131] *Origin of tissue and processing* . Male and female cynomolgus monkeys received subcutaneously SOM230 (100 μg/animal/day) or the vehicle for 14 days. On day 15, all animals were sacrificed and tissues for RNA extraction were immediately snap frozen and kept at –80° C until processing.

TABLE 18
Origin of Tissues Used for Analysis

| Tissue Sample | Animal or sample no. | Sex | Tissue/ organ | Compound | Dose (μg/animal/day) |
|---|---|---|---|---|---|
| x547e | W62405 | Male | Brown fat | SOM230 | 100 |
| x548e | W62406 | Male | Brown fat | SOM230 | 100 |
| x549e | W62425 | Female | Brown fat | SOM230 | 100 |
| x550e | W62426 | Female | Brown fat | SOM230 | 100 |
| x673e | W62401 | Male | Brown fat | Control | 0 |
| x675e | W62421 | Female | Brown fat | Control | 0 |
| x676e | W62422 | Female | Brown fat | Control | 0 |
| x857e | W62501* | Male | Brown fat | Control | 0 |
| x858e | W62502* | Male | Brown fat | Control | 0 |
| x859e | W62551* | Female | Brown fat | Control | 0 |
| x860e | W62552* | Female | Brown fat | Control | 0 |
| d32e | W62551 | Female | Kidney | Control | 0 |
| d35e | W62502 | Male | Kidney | Control | 0 |
| d37e | W62552 | Female | Kidney | Control | 0 |
| d45e | W62501 | Male | Kidney | Control | 0 |
| x407e | W62401 | Male | Kidney | Control | 0 |
| x408e | W62402 | Male | Kidney | Control | 0 |
| x409e | W62421 | Female | Kidney | Control | 0 |
| x410e | W62422 | Female | Kidney | Control | 0 |
| x521e | W62405 | Male | Kidney | SOM230 | 100 |
| x522e | W62406 | Male | Kidney | SOM230 | 100 |
| x523e | W62425 | Female | Kidney | SOM230 | 100 |
| x524e | W62426 | Female | Kidney | SOM230 | 100 |
| x401e | W62401 | Male | Liver left lateral lobe | Control | 0 |
| x402e | W62402 | Male | Liver left lateral lobe | Control | 0 |
| x403e | W62421 | Female | Liver left lateral lobe | Control | 0 |
| x404e | W62422 | Female | Liver left lateral lobe | Control | 0 |
| x517e | W62405 | Male | Liver left lateral lobe | SOM230 | 100 |
| x518e | W62406 | Male | Liver left lateral lobe | SOM230 | 100 |
| x519e | W62425 | Female | Liver left lateral lobe | SOM230 | 100 |
| x520e | W62426 | Female | Liver left lateral lobe | SOM230 | 100 |
| x529e | W62405 | Male | Pancreas | SOM230 | 100 |
| x530e | W62406 | Male | Pancreas | SOM230 | 100 |
| x531e | W62425 | Female | Pancreas | SOM230 | 100 |
| x532-2e | W62426 | Female | Pancreas | SOM230 | 100 |
| x641e | W62401 | Male | Pancreas | Control | 0 |
| x642e | W62402 | Male | Pancreas | Control | 0 |
| x645e | W62421 | Female | Pancreas | Control | 0 |
| x646e | W62422 | Female | Pancreas | Control | 0 |

TABLE 18
Origin of Tissues

| Tissue Sample | Animal or sample no. | Sex | Tissue/ organ | Compound | Dose (µg/animal/day) |
|---|---|---|---|---|---|
| x413e | W62401 | Male | Pituitary gland | Control | 0 |
| x414e | W62402 | Male | Pituitary gland | Control | 0 |
| x415e | W62421 | Female | Pituitary gland | Control | 0 |
| x513-2e | W62405 | Male | Pituitary gland | SOM230 | 100 |
| x514e | W62406 | Male | Pituitary gland | SOM230 | 100 |
| x515e | W62425 | Female | Pituitary gland | SOM230 | 100 |
| x516e | W62426 | Female | Pituitary gland | SOM230 | 100 |
| x425e | W62401 | Male | Spleen | Control | 0 |
| x426e | W62402 | Male | Spleen | Control | 0 |
| x427e | W62421 | Female | Spleen | Control | 0 |
| x428e | W62422 | Female | Spleen | Control | 0 |
| x525e | W62405 | Male | Spleen | SOM230 | 100 |
| x526e | W62406 | Male | Spleen | SOM230 | 100 |
| x527e | W62425 | Female | Spleen | SOM230 | 100 |
| x528e | W62426 | Female | Spleen | SOM230 | 100 |
| d33e | W62501 | Male | Thyroid | Control | 0 |
| d40e | W62551 | Female | Thyroid | Control | 0 |
| d43e | W62502 | Male | Thyroid | Control | 0 |
| d48e | W62552 | Female | Thyroid | Control | 0 |
| x443e | W62401 | Male | Thyroid | Control | 0 |
| x445e | W62421 | Female | Thyroid | Control | 0 |
| x446e | W62422 | Female | Thyroid | Control | 0 |
| x505e | W62425 | Female | Thyroid | SOM230 | 100 |
| x506e | W62426 | Female | Thyroid | SOM230 | 100 |
| x507e | W62405 | Male | Thyroid | SOM230 | 100 |
| x508e | W62406 | Male | Thyroid | SOM230 | 100 |

[00132] RNA expression profiling was conducted by means of the HG-U95A gene expression probe array (Affymetrix; Santa Clara, Calif., USA), containing more than 12,600 probe sets interrogating primarily full-length human genes and also some control probe sets. The assays were conducted according to the recommendations of the manufacturer. Briefly, total RNA was obtained by acid guanidinium thiocyanate-phenol-chloroform extraction (TRIzol®, Invitrogen Life Technologies, San Diego, Calif., USA) from each frozen tissue section. The total RNA was then purified on an affinity resin (Rneasy®, Qiagen) and quantified. Double stranded cDNA was synthesized with a starting amount of approximately 5 µg full-length total RNA using the Superscript® Choice System (Invitrogen Life Technologies, Carlsbad, Calif. USA) in the presence of a T7-(dT)24 DNA oligonucleotide primer. Following synthesis, the cDNA was purified by phenol/chloroform/isoamylalcohol extraction and ethanol precipitation. The purified cDNA was then transcribed in vitro using the BioArray® High Yield RNA Transcript Labeling Kit (ENZO, Farmingdale, New York USA) in the presence of biotinylated ribonucleotides form biotin labeled cRNA. The labeled

cRNA was then purified on an affinity resin (Rneasy®, Qiagen), quantified and fragmented. An amount of approximately 10 µg labeled cRNA was hybridized for 16 hours at 45°C to an expression probe array. The array was then washed and stained twice with streptavidin-phycoerythrin (Molecular Probes, ) using the GeneChip® Fluidics Workstation 400 (Affymetrix, Santa Clara, Calif. USA). The array was then scanned twice using a confocal laser scanner (GeneArray® Scanner, Agilent, Palo Alto, Calif. USA) resulting in one scanned image. This resulting ".dat-file" was processed using the MAS4 program (Affymetrix) into a ".cel-file". The ".cel file" was captured and loaded into the Affymetrix GeneChip® Laboratory Information Management System (LIMS). The LIMS database is connected to a UNIX Sun Solaris server through a network filing system that allows for the average intensities for all probes cells (CEL file) to be downloaded into an Oracle database (NPGN). Raw data was converted to expression levels using a "target intensity" of 150. The data were evaluated for quality control and loaded in the GeneSpring® software 4.2.4 (Silicon Genetics, Calif. USA) for analysis.

[00133] On the human Affymetrix HGU95Av2 chip, probe sets for individual genes contain 20 oligonucleotide pairs, each composed of a "perfect match" 25-mer and a "mismatch" 25-mer differing from the "perfect" match oligonucleotide at a single base. After probe labeling, hybridization, and laser scanning, the expression level was estimated by averaging the differences in signal intensity measured by oligonucleotide pairs of a given probe (AvgDiff value). The fold changes and directions were calculated for selected genes, from the differences of the AvgDiff values between control and treated.

[00134] To identify genes that were impacted by SOM230, the dataset was initially filtered to exclude in a first wave of analysis, genes whose values were systematically in the lower expression ranges where the experimental noise is high (at least 80 in a number of assays corresponding to the smallest number of replicas of any assay point). In a second round of selection a threshold p-value of 0.05 (based on a t-test) identified differences between treated and control based on a two component error model (Global Error Model) and, whenever possible, with a stepdown correction for multi-hypothesis testing (Benjamini and Hochberg false discovery rate). The decision to keep or reject a specific gene was based on the conjunction of numerical changes identified by comparative and statistical algorithms and the relationship to other modulated genes that point to a common biological theme. The weight of this relationship was assessed by the analyst through a review of the relevant scientific literature.

[00135] For the assay analysis described herein: (1) The increase and decrease in expression referred to the RNA expression level unless specifically stated. (2) If there were multiple probe sets representing the same gene, the probe set designed for sense target was favored. (3) The changes in gene expression indicated that a pathway, a cellular activity or component represented by an individual gene might be impacted. Understanding the functional implication is dependent on the information available on the biological context of the transcript level change (gene function, physiological variation, other gene changes, tissue, compound). RT-PCR is used to identify the extent of absolute change in mRNA levels, but this method in general does not add more information on the relevance of the transcript level changes.

[00136] Among the 12,600 genes per chip, about 100 genes were found to reflect the compound signature in a particular tissue. For clarity, they were divided in different classes and subdivided, with many overlaps, into functional categories in the following TABLE.

TABLE 19
SOM230 Gene Expression Profiling

| CLASS | PITUITARY | BROWN FAT | PANCREAS |
|---|---|---|---|
| SIGNAL TRANSDUCTION | | | |
| *1) Phophatidyl inositol and related pathways/PKC, phospho-lipases* | • IP-4-phosphatase, type 1, isoform b ↓ x2<br>• PI-3-kinase,catalytic, α polypeptide ↓ x3<br>• PI-3-kinase, catalytic, δ polypeptide ↓ x2<br>• 1-PI-4-phosphate 5-kinase isoform C ↓ x1.5<br>• PI transfer protein, β ↓ x2.5<br>• PLCγ 1 ↓ x1.5<br>• PKC inhibitor ↑ x2<br>• IP3 receptor, type 1 ↑ x1.5 | • PI-3-kinase. regulatory subunit, polypeptide 2 (p85 β) ↓ x3.5<br>• PI glycan, class F ↓ x2<br>• PLC β 4 ↑ x2<br>• PI glycan, class L ↑ x3.5 | • IP-1-phosphatase ↑ x2.5<br>• PI-4-kinase, catalytic, α polypeptide ↑ x1.5<br>• PL A2, group IVC (cytosolic, calcium-independent) ↑ x1.5 |
| *2) Other calcium/ calcineurin/ calmodulin dependent pathways and associated proteins* | • Calcium/calmodulin-dependent protein kinase I ↑ x2.5<br>• Receptor (calcitonin) activity modifying protein 2 precursor ↑ x3.5 | • Calcium/calmodulin-dependent protein kinase I ↓ x3.5 | |

## TABLE 19
## SOM230 Gene Expression Profiling

| CLASS | PITUITARY | BROWN FAT | PANCREAS |
|---|---|---|---|
| *3) Ras/MAPK kinase/ERK kinase related pathways and adaptor proteins* | • Rab geranylgeranyltransferase, α subunit ↓ x1.5<br>• Rab3 GTPase-activating protein, non catalytic subunit ↓ x2<br>• SHB adaptor protein (a Src homology 2 protein) ↓ x2.5<br>• MAPKKK5 ↑ x2<br>• Rab geranyltransferase, β subunit ↑ x2<br>• RAB 5C, member RAS oncogene family ↑ x3 | • Ras homolog gene family, member G (rho G)<br>• MAPKAPK 3<br>• MAPKK1<br>• MAPK 8<br>• RAB6, member RAS oncogene family<br>• Adaptor-related protein complex 3, σ 1 subunit<br>• Ras-related nuclear protein<br>• Rab acceptor 1 (prenylated)<br>• RAB 2, member RAS oncogene family<br>• IQ motif containing GTPase activating protein 2 | • SH3 domain binding glutamic acid-rich protein<br>• MAPKAPK 3<br>• Ras like GTPase<br>• RaP2 interacting protein 8 |
| *4) JAK/STAT pathway and related kinases* | • STAT 1, 91kδ ↓ x2<br>• JAK 1 ↑ x2 | • JAK 3 | • STAT 5B<br>• STAT 1, 91kδ<br>• STAT 2 |
| *5) Protein tyrosine phosphatases/other phosphatases* | • Dual specificity phosphatase 8 ↓ x3<br>• Phosphatase and tensin homolog (mutated in multiple advanced cancers 1) ↓ x3.5<br>• PTP, receptor type, T ↑ x2<br>• PP 1, regulatory (inhibitor) subunit 5 ↑ x3.5 | • PP 2, regulatory subunit B (B56), γ isoform ↓ x1.5<br>• PP 5, catalytic subunit ↑ x2.5<br>• Dual specificity PP MKP-5 ↑ x2.5 | • PTP δ<br>• PP 1, regulatory (inhibitor) subunit 8<br>• PP 2A, catalytic subunit B'<br>• PP 1A (formerly 2C), magnesium-dependent, α isoform<br>• PP 2A, regulatory subunit B'<br>• Dual specificity phosphatase 8 |
| *6) Other protein kinases and associated binding proteins* | • Arg PTK-binding protein ↓ x2.5 | • PTK 9-like (A6-related protein)<br>• PTK A kinase (PRKA) anchor protein 1<br>• cAMP-dependent protein kinase R1-β regulatory subunit<br>• Ribosomal protein S6 kinase, 90kD, polypeptide | • Protein kinase (c AMP-dependent, catalytic), inhibitor γ<br>• Serine/threonine protein kinase<br>• Receptor PTK<br>• Serine/threonine kinase 11 (Peutz-Jeghers syndrome)<br>• Tyrosine kinase<br>• Ribosomal protein S6 kinase, 90kδ, polypeptide 3 |

TABLE 19
SOM230 Gene Expression Profiling

| CLASS | PITUITARY | BROWN FAT | PANCREAS |
|---|---|---|---|
| *7) Adenylate/guanylate cyclases and related pathways* | • Soluble adenylyl cyclase ↓ x2 | | |
| CELL SURFACE RECEPTORS | | | |
| *1) G-protein coupled receptors and related binding proteins/ G proteins* | • GTP-binding protein (G protein), q polypeptide ↓ x2.5<br>• GTP-binding protein like-1 ↓ x3<br>• G-protein coupled receptor 49 ↓ x2<br>• G protein-coupled receptor, family C, group 5, member B ↑ x2<br>• GTP-binding protein 11 ↑ x2.5<br>• Receptor tyrosine kinase-like orphan receptor 2 ↑ x2<br>• ATP(GTP)-binding protein ↑ x1.5<br>• G-protein coupled receptor 9 ↑ x2.5<br>• Regulator of G-protein signaling 9 ↑ x2<br>• SSTR3 ↑ x3 | • G α inhibiting activity polypeptide 3 interacting protein ↓ x5<br>• G protein-coupled receptor 1 ↓ x2.5<br>• Guanine nucleotide binding protein (G protein), β polypeptide 3 ↑ x2.5<br>• SSTR3↑ x6.5<br>• Endothelial differentiation, sphingolipid G-protein-coupled receptor, 5 ↑ x2.5 | • G protein-coupled receptor 39<br>• G protein-coupled receptor 49<br>• G protein-coupled receptor 3<br>• Regulator of G-protein signaling 10<br>• GTP-binding protein<br>• SSTR2 ↓ x1.5 |

## TABLE 19
## SOM230 Gene Expression Profiling

| CLASS | PITUITARY | BROWN FAT | PANCREAS |
|---|---|---|---|
| *2) Growth factors, their receptors and related binding proteins* | • FGFR 2 ↓ x2<br>• EGFRBP 2 ↓ x1.5<br>• Fms-related tyrosine kinase 1 (VEGF/vascular permeability factor receptor) ↓ x1.5<br>• Catenin (cadherin-associated protein), α 1 (102kD) ↓ x1.5<br>• PDGFβ ↓ x2<br>• GFR bound protein 10 ↑ x1.5<br>• Butyrate response factor 2 (EGF-response factor 2) ↑ x3<br>• VEGF B ↑ x1.5 | • Fms-related tyrosine kinase 1 (VEGF/ vascular permeability factor receptor) ↓ x4.5<br>• EGF receptor pathway substrate 15 ↓ x2<br>• CSF 1 (macrophage) ↓ x2<br>• Cadherin 13, H-cadherin (heart) ↓ x2<br>• Cadherin F1B1 ↓ x4<br>• Endothelial cell GF 1 (platelet derived) ↓ x2<br>• TGF β -activated kinase-binding protein 1 ↑ x2.5<br>• CSF 3 receptor (granulocyte) ↑ x3<br>• TGF β 3 ↑ x2.5<br>• Cadherin 5, VE-cadherin (vascular epithelium) ↑ x3<br>• VGF nerve growth factor inducible ↑ x2<br>• IL 3 (CSF, multiple) ↑ x2<br>• IL 7R precursor ↑ x2 | • Smad 3 ↓ x1.5<br>• G-CSF protein ↓ x2<br>• PDGFR α ↑ x2.5<br>• PDGFR, α polypeptide ↑ x1.5 |
| *3) Glutamate receptor and related binding proteins* | • GLUR 2, precursor ↑ x1.5 | • GLUR, metabotropic 1 ↑ x2 | • GLUR precursor, flip isoform ↑ x3 |

TABLE 19
SOM230 Gene Expression Profiling

| CLASS | PITUITARY | BROWN FAT | PANCREAS |
|---|---|---|---|
| ATP-DEPENDENT TRANSPORT PROTEINS<br>*Ion channels and related pathways* | • K+ channel, subfamily K, member 3 (TASK) ↓ x4<br>• K+ voltage-gated channel, Shab-related subfamily, member 1 ↓ x2<br>• ATPase, H+/K+ exchanging, α polypeptide ↓ x5<br>• ATPase, Na+/K+ transporting, α 2 (+) polypeptide ↑ x2.5<br>• ATPase, Na+/K+ transporting, β 3 polypeptide ↑ x2.5<br>• ATPase, Ca++ transporting cardiac muscle, slow twitch 2 ↑ x1.5<br>• Putative Ca++ transporting ATPase ↑ x2 | • Ca++ channel, voltage dependent, α 1H subunit ↑ x3<br>• G protein-activated inwardly rectifying K+ channel ↑ x3.5 | • K+ voltage-gated channel, KQT-like subfamily, member3 ↓ x2.5<br>• Ca++ channel, voltage dependent, α 1F subunit ↓ x4.5<br>• Na+ channel, voltage-gated, type I, β polypeptide ↑ x2 |
| CELL BIOLOGY/ SPECIALIZED FUNCTIONS<br>*1) Neuromediators/ neuromodulators and related pathways* | • Cholinergic receptor,nicotinic, β polypeptide 4 ↓ x2<br>• Cholinergic receptor, muscarinic 3 ↓ x3<br>• Brain cannabinoid receptor 1 ↑ x2<br>• GABA-B R 1, isoform a precursor ↑ x1.5 | • Dopamine receptor D3 ↑ x2.5<br>• Adrenergic, β-3-, receptor ↑ x2.5 | |
| *2)Pancreatic/ gastro-intestinal secretions and related pathways* | • Cholecystokinin receptor ↓ x4.5<br>• Gastrin receptor ↓ x2 | | • Chymotrypsin-like ↑ x3.5<br>• Gastrin-releasing peptide receptor ↓ x5 |

## TABLE 19
## SOM230 Gene Expression Profiling

| CLASS | PITUITARY | BROWN FAT | PANCREAS |
|---|---|---|---|
| *3) Hormones and related pathways* | • IGF-2 ↓ x1.5<br>• Thyroid transcription factor 1 ↑ x2.5<br>• Glucagon receptor ↑ x7<br>• IGFBP, acid labile subunit ↑ x3.5<br>• Adrenomedullin ↑ x2.5<br>• ANP (atrial natriuretic peptide precursor B) ↑ x2<br>• SSTR3 ↑ x3 | • THR interactor 10 ↓ x3<br>• THR interactor 12 ↓ x1.5<br>• IGF-1 ↓ x1.5<br>• IGF-binding protein 4 ↓ x2<br>• IRS (insulin receptor substrate) 2 ↑ x2.5<br>• T3 receptor ↑ x2<br>• SSTR3 ↑ x6.5<br>• Oxytocin, prepro- (neurophysin I) ↑ x2.5<br>• FSHR ↑ x2.5 | • CRHR 1 ↓ x2<br>• THR binding protein ↓ x2<br>• THR interactor 10 ↑ x2.5<br>• IGF-1 ↑ x4.5<br>• Prostacyclin synthase ↑ x2<br>• SSTR2 ↓ x1.5 |
| *4) Cytoskeletton and associated proteins* | • Thrombospondin-p50 ↓ x2<br>• CD36 antigen ↓ x2 | • Capping protein (actin filament), gelsolin-like ↓ x2.5<br>• Actin related protein 2/3 complex, subunit 1A (41kD) ↓ x2.5 | • Integrin α 2b precursor ↑ x1.5 |
| *5) Enzymes* | | • Coagulation factor XIIIAI subunit precursor ↓ x5 | • Thrombospondin 2 ↑ x3.5 |
| IMMUNITY | | | |
| | • TNFR-associated factor 2 ↓ x4<br>• TNFR subfamily, member 14; herpesvirus entry mediator ↓ x2.5<br>• IFNR2 (α, β and ω) ↓ x2.5<br>• CC chemokines STCP-1 ↑ x1.5<br>• IFN stimulated gene ↑ x3.5<br>• IFNγ-inducible protein 30 (IP30) ↑ x1.5 | • IFN γ-inducible protein 30 (IP30) ↓ x3.5<br>• Pentaxin-related gene, rapidly induced by IL-1 ↓ x7.5<br>• IFN induced transmembrane protein 1 ↑ x2.5<br>• TNF type 1 receptor associated protein ↓ x2<br>• IL 5R, α↑ x2.5<br>• CD2 antigen (cytoplasmic tail)-binding protein 2 ↑ x2.5 | • IL 1 receptor antagonist↓ x2<br>• LT b4 receptor (chemokine receptor like-1) ↓ x1.5<br>• Phosphotyrosine independent ligand p62B for the Lck SH2 domain B-cell isoform ↓ x2<br>• IFN regulatory factor 3 ↑ x5 |

## TABLE 19
## SOM230 Gene Expression Profiling

| CLASS | PITUITARY | BROWN FAT | PANCREAS |
|---|---|---|---|
| CELL CYCLE | • Forkhead box O3A ↓ x1.5<br>• Cyclin F ↓ x2<br>• Core-binding factor, runt domain, α subunit 2; translocated to, 1; cyclin D-related ↑ x3<br>• S-phase response (cyclin-related) ↑ x2<br>• Cell division cycle 25B ↑ x5<br>• Cyclin D3 ↑ x2.5<br>• Cdki2C (p18, inhibits CDK4) ↑ x2<br>• Cdki2D (p19, inhibits CDK4) ↑ x1.5<br>• Forkhead box H1↑ x2 | • G1 to S phase transition ↓ x1.5<br>• Extra spindle poles, S.cerevisiae, homolog of ↓ x2.5<br>• PCNA ↓ x2.5<br>• Follistatin-like 3 glycoprotein ↑ x3<br>• Cyclin T2 ↑ x2.5 | • Cyclin T2 ↓ x2<br>• Cyclin D1 ↑ x2<br>• Cdki 1C ↑ x2 |
| APOPTOSIS | • BCL2-associated athanogene ↑ x2<br>• BCL2-antagonist of cell death ↑ x2<br>• Bax γ ↑ x1.5<br>• BCL2/adenovirus E1B 19kD-interacting protein 3 ↑ x1.5<br>• Programmed cell death 6 ↑ x1.5<br>• Neuroblastoma-amplified protein ↑ x1.5 | • Neuroblastoma ↓ x2.5<br>• Neuroblastoma apoptosis-related RNA bindingprotein ↓ x3<br>• Apotosis-associated tyrosine kinase ↑ x3.5 | • BCL2/adenovirus E1B 19kD-intracting protein 1, isoform BNIP1-a ↓ x1.5<br>• Neuroblastoma apoptosis-related RNA binding protein ↓ x3 |

TABLE 20
SOM230 Gene Expression Profiling (Continued)

| CLASS | KIDNEY | LIVER | SPLEEN | THYROID |
|---|---|---|---|---|
| SIGNAL TRANSDUCTION<br>*1) Phophatidyl inositol and related pathways/PKC, phospholipases* | • PI-3- kinase, catalytic, α polypeptide ↓ x3 | • PI transfer protein, β ↓ x1.5<br>• 1- PI-4-phosphate 5-kinase isoform C ↓ x2<br>• Glycosylphosphatidylinositol specific phospholipase D1 ↓ x1.5<br>• PKC, ι ↓ x1.5<br>• PLC, γ 1 (formerly subtype 148) ↑ x2<br>• PKC substrate 80K-H ↑ x1.5<br>• PLA2, group IIA (platelets, synovial fluid) ↑ x5.5<br>• PI transfer protein ↑ x3.5<br>• Nck, Ash and PLC γ binding protein NAP4 ↑ x3.5<br>• DAG kinase, α (80kD) ↑ x3<br>• DAG kinase, δ (130kD) ↑ x1.5<br>• IP 5-phosphatase ↑ x2 | • PI-3-kinase, catalytic, α polypeptide ↓ x2.5<br>• PI-3- kinase, class 3 ↑ x2<br>• PLA2 ↑ x2<br>• PKC, α binding protein ↑ x2<br>• IP-4-phosphatase, type 1, isoform b ↑ x2<br>• PI-3-kinase, class 2, β polypeptide ↑ x1.5<br>• Phosphatidyl inositol glycan, class B ↑ x1.5 | • IP3 receptor type 3 ↓ x1.5<br>• PLC, γ 1 (formerly subtype 148) ↓ x2<br>• PIP 5-phosphatase type IV ↓ x3<br>• DAG 1 kinase, α (80kD) ↓ x4 |
| *2) Other calcium/ calcineurin/ calmodulin dependent pathways and associated proteins* | • PP 3 (formerly 2B), catalytic subunit, β isoform (calcineurin A β) ↓ x2<br>• Calmodulin 3 (phosphorylase kinase, δ) ↓ x1.5<br>• Calcium/ calmodulin-dependent protein kinase kinase 2 β ↑ x1.5 | • Calcium/ calmodulin-dependent protein kinase kinase 2, β ↑ x3<br>• Calmodulin 2 (phosphorylase kinase, δ) ↑ x2<br>• Receptor (calcitonin) activity modifying protein 1 precursor ↑ x1.5 | • Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 ↓ x5<br>• Calmodulin 1 (phosphorylase kinase, δ) ↑ x2<br>• Calmodulin 2 (phosphorylase kinase, δ) ↑ x1.5<br>• Calcium/ calmodulin-dependent protein kinase (CaM kinase) II β ↑ x2 | • FKBP-associated protein ↓ x2.5<br>• Calmodulin-dependent PK IV (CaM-kinase IV) ↓ x7.5<br>• Calcium/ calmodulin-dependent PK IV ↓ x7.5<br>• c-AMP responsive element binding protein 1 ↓ x2<br>• Calcium/ calmodulin dependent protein kinase 1 ↓ x2 |

TABLE 20
SOM230 Gene Expression Profiling (Continued)

| CLASS | KIDNEY | LIVER | SPLEEN | THYROID |
|---|---|---|---|---|
| *3) Ras/MAPK kinase/ERK kinase related pathways and adaptor proteins* | • Ras suppressor protein 1<br>• Rho GTPase activating protein 4<br>• MAPKK 5<br>• Rho GTPase activating protein 5<br>• RAB4, member RAS oncogene family<br>• RAB interacting factor<br>• Realted RAS viral (r-ras) oncogene homolog<br>• RAP2A, member of RAS oncogene family<br>• Human rho GDP-dissociation inhibitor<br>• MAPKK 1 | • Ras association (RalGDS/AF-6) domain family 1<br>• Rho GDP dissociation inhibitor (GDI) γ<br>• Rab geranylgeranyl-transferase, α subunit<br>• MAPK 10<br>• RAB13, member RAS oncogene family<br>• Ras homolog gene family, member G (rho G)<br>• RAB2, member RAS oncogene family<br>• MAPK 1<br>• C-src tyrosine kinase<br>• MAPK 14<br>• Rho GTPase-activating protein 1<br>• MAPKK 1<br>• ATP(GTP)-binding protein<br>• RAB interacting factor<br>• MAPK 6<br>• KAPKK 5<br>• RAB 30, member RAS oncogene family<br>• RAB 4, member RAS oncogene family<br>• MAPKK 13<br>• MAP/ERK kinase kinase 4, isoform a | • Rho/rac guanine nucleotide exchange factor (GEF) 2<br>• Rab geranylgeranyl-transferase<br>• Human rho GDP-dissociation inhibitor 2 (IEF 8120)<br>• SHP2 interacting transmembrane adaptor<br>• RAS p21 protein activator (GTPase activating protein) 1<br>• SH3 domain binding glutamic acid-rich protein like<br>• Neuronal shc<br>• MAPKK 1<br>• MAKKK 5<br>• RAB11B, member of RAS oncogene family<br>• GTPase<br>• RAB5B, member RAS oncogene family<br>• MAPKAPK 2<br>• MAPK 6<br>• Ras-related C3 botulinum toxin substrate 1 isoform Rac 1b<br>• RAB1, member ras oncogene family<br>• RAP1A, member of ras oncogene family<br>• MAPKKKK<br>• RAS guanyl releasing protein 2 (calcium and DAG-regulated)<br>• Grb2-associated binder 2 | • Ras homolog gene family, member B<br>• Ras-GTPase activating protein •<br>SH3 domain-binding protein 2<br>• Rho-associated, coiled-coil containing protein kinase 1<br>• RAD54 (S.cerevisiae)-like<br>• RAB6, member RAS oncogene family<br>• RAB5A, member RAS oncogene family<br>• MAPKK 4<br>• SH3 domain binding glutamic acid-rich protein<br>• MAPK 8<br>• Adaptor protein with pleckstrin homology and src homology 2 domains<br>• SHP2 interacting transmembrane adaptor<br>• Ras homolog gene family, member H<br>• RaP2 interacting protein 8<br>• RAB5C, member RAS oncogene family<br>• Grb2-associated binder 2 |

TABLE 20
SOM230 Gene Expression Profiling (Continued)

| CLASS | KIDNEY | LIVER | SPLEEN | THYROID |
|---|---|---|---|---|
| *4).JAK/STAT pathway and related kinases* | • STAT 2, 113kD<br>• STAT 5B | • STAT 1, 91kδ | • JAK 3 | • JAK 1<br>• STAT 6, IL-4 induced<br>• Protein inhibitor of STATX<br>• STAT 1, 91kδ<br>• STAT 3 (acute-phase response factor) |
| *5) Protein tyrosine phosphatases/other phosphatases* | • PP 1, regulatory subunit 7<br>• PP 1A (formerly 2C), Mg-dependent, α isoform<br>• PTP<br>• PP 2A, regulatory subunit B' (PR 53)<br>• PP 2A, regulatory subunit -β<br>• PTP, non- receptor type 1<br>• PTP type IVA, member 3<br>• PP5, catalytic subunit | • PTP<br>• PP 2 (formerly 2A), regulatory subunit A (PR 65), α Isoform<br>•PP2A subunit- α<br>• PTP, non receptor type 1<br>•PTP, non receptor type substrate 1<br>• PP 6, catalytic subunit<br>• PTP, receptor type, C<br>• PP 1, regulatory (inhibitor) subunit 5<br>• PTP, receptor type, f polypeptide (PTPRF), interacting protein (liprin), α 1 | • Dual specificity phosphatase 8<br>• Myosin phosphatase target subunit 1<br>• Dual specificity phosphatase 9<br>• PTP, non- receptor type 1<br>• PP 1, regulatory (inhibitor) subunit 8<br>• PTP, receptor type, N<br>• PTP type IVA, member 3<br>• PP 5, catalytic subunit<br>• PTP σ | • PTP σ<br>• Phosphatase and tensin homolog 2<br>• PP 5, catalytic subunit<br>• PTP, non-receptor type 6<br>• PP 1A (formerly 2C), Mg- dependent, α isoform<br>• PTP, receptor type, C<br>• PTP, receptor type, N<br>• Phosphatidid acid phosphatase type 2A<br>• PTP, receptor type, A |

TABLE 20
SOM230 Gene Expression Profiling (Continued)

| CLASS | KIDNEY | LIVER | SPLEEN | THYROID |
|---|---|---|---|---|
| *6) Other protein kinases and associated binding proteins* | • Receptor tyrosine kinase<br>• Protein kinase<br>• Serine/threonine kinase 3<br>• SNF1-like protein kinase<br>• Serine/threonine kinase 9<br>• Membrane-associated kinase<br>• Ser-Thr protein kinase related to the myotonic dystrophy protein kinase<br>• cAMP-dependent protein kinase<br>• RI-β regulatory subunit<br>• Ribosomal protein S6 kinase, 90kδ, polypeptide 4<br>• Serine/threonine kinase 25<br>• Fms-related tyrosine kinase 3 | • Protein kinase, cAMP-dependent, catalytic, inhibitor α<br>• Tyrosine kinase 2<br>•Protein kinase<br>•PTK2 protein tyrosine kinase 2<br>• Ribosomal protein S6 kinase, 90kδ, polypeptide 3<br>• Protein kinase, cAMP-dependent, catalytic, γ<br>• Serine threonine protein kinase | • Serine/threonine kinase 14 α<br>• Serine/threonine kinase<br>• Protein kinase, AMP-activated, γ 1 non-catalytic subunit<br>• Protein kinase, cAMP-dependent, catalytic inhibitor α<br>• Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A<br>• Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2 isoform 1<br>• Serine/threonine kinase 19 | • Serine kinase<br>• Serine/threonine kinase 25<br>• Serine/threonine protein kinase<br>• Ste-20 related kinase<br>• Ribosomal protein S6 kinase, 90kδ, polypeptide 3<br>• Serine/threonine kinase 13 (aurora/IPL1-like)<br>• Protein-tyrosine kinase<br>• Membrane-associated kinase<br>• Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2 isoform 1 |
| *7) Adenylate/guanylate cyclases and related pathways* | • Natriuretic peptide receptor A/guanylate cyclase A (atrionatriuretic peptide receptor A) ↑ x2 | • Natriuretic peptide receptor A/ guanylate cyclase A (atrionatriuretic peptide receptor A) ↑ x6<br>• Adenylyl cyclase-associated protein ↑ x1.5 | | • Adenylate cyclase activating polypeptide precursor ↓ x1.5 |

TABLE 20
SOM230 Gene Expression Profiling (Continued)

| CLASS | KIDNEY | LIVER | SPLEEN | THYROID |
|---|---|---|---|---|
| CELL SURFACE RECEPTORS<br>*1) G-protein coupled receptors and related binding proteins/G proteins* | • Guanine nucleotide binding protein (G protein), β polypeptide 1<br>• G protein-coupled receptor 20<br>• G protein-coupled receptor 9<br>• G protein-coupled receptor 39<br>• G protein-coupled receptor kinase<br>• G protein-coupled receptor 15<br>• Guanine nucleotide binding protein (G protein), β polypeptide 2<br>• G protein-coupled receptor 35<br>• SSTR 3↑ x3<br>• SSTR 2↑ x2 | • G protein-coupled receptor 12<br>• G protein-coupled receptor kinase<br>• G protein-receptor coupled 35<br>• G protein-coupled receptor 3<br>• G protein-receptor coupled 39<br>• Regulator of G-protein signalling 6<br>• Coagulation factor II (thrombin) receptor-like 1 precursor | • Guanine nucleotide binding protein 11<br>• Guanine nucleotide binding protein (G protein), β polypeptide 3<br>• G protein-coupled receptor 56<br>• Regulator of G-protein signalling 9<br>• Guanine nucleotide binding protein (G protein), α 11 (Gq class)<br>• G protein-coupled receptor 35<br>• Angiotensin receptor-like 1 ↑ x1.5<br>• SSTR2 ↑ x2<br>• GDP dissociation inhibitor | • Guanine nucleotide binding protein (G protein), α 15 (Gq class)<br>• G α inhibiting activity polypeptide 3 interacting protein<br>• Regulator of G protein signaling<br>• Guanine nucleotide binding protein 11<br>• G protein – coupled receptor 3<br>• G protein – coupled receptor kinase 1<br>• Ca++-sensing receptor (hypocalciuric hypercalcemia 1, severe neonatal hyperparathyroid ism)<br>• G protein-coupled receptor, family C, group 5, member B<br>• Guanine nucleotide binding protein 11<br>• 5-hydroxytryptamine 7 receptor isoform b<br>• Developmentally regulated<br>• GTP-binding protein 2<br>• Endothelial differentiation-related factor 1 ↑ |

TABLE 20

SOM230 Gene Expression Profiling (Continued)

| CLASS | KIDNEY | LIVER | SPLEEN | THYROID |
|---|---|---|---|---|
| *2) Growth factors, their receptors and related binding proteins* | • GFR- bound protein 7 ↑ x2<br>• GFR- bound protein 14 ↑ x2<br>• CSF-1 receptor, formerly Mc Donough feline sarcoma viral (v-fms) oncogene homolog ↑ x2.5<br>• IL-7 R precursor ↑ x2<br>• PDGFR, α polypeptide ↑ x1.5<br>• TGF, β 1 ↑ x1.5 | • Fms-related tyrosine kinase 1(VEGF /vascular permeability factor receptor) ↓ x1.5<br>• GFR- bound protein 2 ↓ x2.5<br>• Bone-derived GF ↑ x3<br>• Growth differentiation factor 1 ↑ x3<br>• TGF, β1 ↓ x1.5<br>• TGFβR III (betaglycan, 300kδ) ↓ x2<br>• EGF (β-urogastrone) ↓ x2.5<br>• EGFR (avian erythroblastic leukemia viral (v-erb-b) oncogene homolog) ↑ x2<br>• Butyrate response factor 2 (EGF-response factor 2) ↑ x2<br>• FGFR 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysplasia) ↑ x2<br>• TGFβ activated kinase-binding protein 1 ↑ x2.5<br>• GCSF ↑ x3.5<br>• EGF-like repeats and discoidin I-like domains 3 ↓ x1.5<br>• PDGFR, α polypeptide ↑ x2<br>• PDGF, α polypeptide ↑ x1.5 | • EGF (β-urogastrone) ↓ x2<br>• TGF induced protein ↑ x1.5<br>• VEGF ↑ x1.5<br>• PDGF α polypeptide ↑ x2.5<br>• PDGFR, α polypeptide ↑ x1.5<br>• TGFβ receptor III (betaglycan, 300kD) ↓ x1.5<br>• HGF activator inhibitor precursor ↑ x1.5 | • COL1A1 and PDGFB fusion transcript ↓ x6<br>• Egf-like module containing, mucin-like, hormone receptor-like sequence 1 ↓ x5<br>• PDGFR α ↓ x3<br>• Fms-related tyrosine kinase 1 (VEGF/ vascular permeability factor receptor) ↓ x2<br>• PDGF-associated Protein ↑ x2<br>• PDGFR β ↑ x2<br>• Cadherin 13, H-cadherin (heart) ↑ x2 |

TABLE 20
SOM230 Gene Expression Profiling (Continued)

| CLASS | KIDNEY | LIVER | SPLEEN | THYROID |
|---|---|---|---|---|
| *3) Glutamate receptor and related binding proteins* | • Glutamate receptor metabotropic 2 precursor ↓ x3 | • Glutamate receptor, metabotropic 4 ↑ x1.5 | | • Glutamate receptor, metabotropic 2 precursor ↓ 3.5 |
| ATP-DEPENDENT TRANSPORT PROTEINS | | | | |
| *Ion channels and related pathways* | • Solute carrier family 6 (neurotransmitter transporter, creatin), member 8 ↓ x3<br>• Na+ channel, nonvoltage-gated 1, β (Liddle syndrome) ↓ x2<br>• Ca++ channel, voltage-dependent, α 1H subunit ↑ x2<br>• K+ voltage-gated channel, Shaw-related subfamily, member 3 ↑ x2.5<br>• Solute carrier family 9 (Na+/H+ exchanger) isoform 3 regulatory factor 1 ↑ x2 | • Ca++ channel, voltage-dependent, P/Q type, alpha 1A subunit ↓ x2.5<br>• ATPase, H+/K+ exchanging, beta polypeptide ↓ x2<br>• Solute carrier family 9 (sodium/ hydrogen exchanger), isoform 3 regulatory factor 1 ↑ x11.5<br>• Solute carrier family 11 (Na+/phosphate symporters), member 1 ↑ x2.5 | • K+ voltage-gated channel, shaker-related subfamily, member 3 ↓ x3<br>• Solute carrier family 9 (Na+/H+ exchanger) isoform 3 regulatory factor 1 ↑ x10.5<br>• ATPase, Na+/K+ transporting, β 1 polypeptide ↑ x2.5<br>• K+ large conductance Ca++-activated channel, subfamily M, β member 1 ↑ x2.5<br>• Ca++ channel, voltage-dependent, α 2/δ subunit 2 ↑ x1.5<br>• Ca++ channel, voltage- dependent, α 2/δ subunit 1 ↑ x2 | • K+ voltage-gated channel, shaker-related subfamily, member 3 ↓ x4.5<br>• ATPase, Ca++ transporting, cardiac muscle, fast twitch 1 ↓ x4.5 |

TABLE 20
SOM230 Gene Expression Profiling (Continued)

| CLASS | KIDNEY | LIVER | SPLEEN | THYROID |
|---|---|---|---|---|
| CELL BIOLOGY/ SPECIALIZED FUNCTIONS *1) Neuromediators/ neuromodulators and related pathways* | • δ sleep inducing peptide, immunoreactor↑ x2.5<br>• Opioid receptor, δ1 ↑ x2.5<br>• GABA (A) receptor, γ2 precursor ↑ x2<br>• Acetylserotonin O-methyl transferase-like ↓ x3<br>• LIF (cholinergic differentiation factor) ↑ x2<br>• Dopamine receptor D2 ↑ x4.5 | • GABA (A) receptor, γ2 precursor ↑ x4<br>• Dopamine receptor D2 ↑ x3.5<br>• GABA(A) receptor-associated protein ↑ x1.5<br>• Dopamine receptor D3 ↑ x2<br>• δ sleep inducing peptide, immunoreactor ↑ x2.5<br>• 5-hydroxytrypt amine (serotonine) receptor 6 ↑ x2.5 | • Dopamine receptor D4 ↓ x2<br>• Adrenergic α-2C-receptor ↓ x2<br>• β adrenergic receptor kinase 1 ↑ x3 | • GABA (A) receptor, γ 2 precursor ↓ x1.5<br>• Brain cannabinoid receptor 1 ↓ x2<br>• GABA (B) receptor 1, isoform a precursor ↑ x2.5<br>• Cannabinoid receptor 2 (macrophage) ↑ x3<br>• Phosphatidyl ethanolamine N-methyltransferase ↑ x2<br>• Adrenergic, α -2C-, receptor ↓ x2.5<br>• Cholecystokinin B receptor ↓ x3<br>• Gastric inhibitory polypeptide 1 receptor ↓ x2 |
| *2) Pancreatic/ gastro-intestinal secretions and related pathways* | | | • Gastric inhibitory polypeptide receptor ↑ x2 | |

TABLE 20
SOM230 Gene Expression Profiling (Continued)

| CLASS | KIDNEY | LIVER | SPLEEN | THYROID |
|---|---|---|---|---|
| *3) Hormones and related pathways* | • Angiotensin receptor 1B ↓ x1.5<br>• Glucocorticoid receptor DNA binding factor 1 ↓ x4.5<br>• Insulin receptor ↓ x3<br>• THR, α (avian erythroblastic leukemia viral (v-erb-a) oncogene homolog) ↑x1.5<br>• Arginine vasopressin (neurophysin II, antidiuretic hormone, diabete insipidus, neurohypophyseal) ↑ x2<br>• Vasopressin-activated calcium-mobilizing receptor-1 ↓ x2<br>• Corticotropin releasing hormone receptor type 2 beta isoform ↑ x1.5<br>• IGF-2 ↓ x2<br>• IGF-1 ↓ x2.5<br>• IGFBP2 ↑ x1.5<br>• THR-associated protein, 240kδ subunit ↓ x1.5<br>• THR binding protein ↑x1.5<br>• PG-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase) ↓ x3.5<br>• Adrenomedullin ↑ x1.5<br>• SSTR 3↑ x3<br>• SSTR 2↑ x2 | • Insulin promoter factor 1, homeodomain transcription factor ↓ x1.5<br>• IGF-2 ↓ x2.5<br>• Corticosteroid binding globulin precursor ↑ x2<br>• THR interacting protein 15 ↑ x1.5<br>• IGFBP2 ↑ x2<br>• Arginine vasopressine receptor 2 ↑ x2.5<br>• THR sulfo transferase ↑ x2.5<br>• Glucacon receptor ↑ x5 | • PTHR 1 ↓ x2.5<br>• Arginine vasopressine receptor 1B ↑ x2<br>• IGFBP6 ↑ x2.5<br>• IGF-1 ↑ x1.5 | • TSHR ↓ x2<br>• IGF-1 ↓ x1.5<br>• Solute carrier family 21 (PG transporter), member 2 ↑ x2 |

TABLE 20
SOM230 Gene Expression Profiling (Continued)

| CLASS | KIDNEY | LIVER | SPLEEN | THYROID |
|---|---|---|---|---|
| *4) Cytoskeletton and associated proteins* | • VWF precursor ↑ x2 | | • Vasodilatator-stimulated phosphoprotein ↑ x2 | • VWF precursor ↑ x2 |
| *5) Enzymes* | | | | • Thrombospondin 2 ↑ x2<br>• Pro-platelet basic protein (includes platelet basic protein, β-thromboglobulin, connective tissue-activating peptide III, neu) 2 ↑ x3.5 |
| IMMUNITY | • TNF, α-induced protein 3 ↓ x1.5<br>• IRF5 ↑ x2<br>• Putative chemokine receptor; GTP-binding protein ↑ x2<br>• TNFR superfamily, member 12 ↑ x2 | • TNF-α converting enzyme ↓ x2<br>• IFN-stimulated protein, 15kDa ↓ x2<br>• IFN-related developmental regulator 2 ↓ x1.5<br>• IFN-inducible RNA-dependent protein kinase ↑ x4<br>• IL2 R, γ chain, precursor ↑ x2.5<br>• IFN regulatory factor 5 ↑ x2.5<br>• Bruton agammaglobulinemia tyrosine kinase ↑ x1.5<br>• B lymphoid tyrosine kinase ↑ x3.5<br>• IFN γ responsive transcript ↑ x1.5<br>• TNF (ligand) superfamily, member 10 ↑ x1.5<br>• IFN-induced leucine zipper protein ↑ x1.5 | • IFN-inducible RNA-dependent protein kinase ↑ x2.5<br>• TNF (cachectin) ↑ x2<br>• TNF (ligand) superfamily, member 13 ↑ x2.5<br>• IL 1 receptor- like 1 ↑ x2<br>• IFNγ responsive transcript ↑ x2<br>• LTb4 (chemokine rceptor-like 1) ↑ x3<br>• Putative chemokine receptor; GTP-binding protein ↑ x2.5<br>• IFNγ receptor 2 ↑ x1.5 (IFNγ transducer 1)<br>• TNFR superfamily, member 12 ↑ x1.5<br>• IL-8 receptor type B ↑ x1.5<br>• IFN regulatory factor 2 ↑ x3.5 | • LTB4 receptor (chemokine receptor-like 1) ↓ x1.5<br>• IL2-inducible T-cell kinase ↓ x12<br>• P56lck ↓ x18<br>• RAG1 ↓ x18<br>• IFNγ responsive transcript ↓ x1.5<br>• SH2 domain protein 1A, Duncan's disease (lymphoproliferative syndrome) ↓ x7.5<br>• CD2 antigen (p50), sheep red blood cell receptor ↓ x7.5<br>• TCR ζ chain precursor ↓ x5.5<br>• RAG2 ↓ x5<br>• Signaling lymphocytic activation molecule ↓ x4.5<br>• Flt3 ligand ↓ x4.5<br>• Lymphocyte specific protein tyrosine kinase ↓ x4 |

TABLE 20

SOM230 Gene Expression Profiling (Continued)

| CLASS | KIDNEY | LIVER | SPLEEN | THYROID |
|---|---|---|---|---|
| IMMUNITY (Continued) | | | | • Chemokine (C-X-C motif), receptor 4 (fusin) ↓ x3<br>• Transcription factor 7 (T-cell specific, HMG-box) ↓ x16.5<br>• IL9 receptor ↓ x2<br>• RANTES ↑ x10.5<br>• CD2 antigen (cytoplasmic tail)-binding protein 2 ↑ x3.5<br>• IFNγ - inducible protein 30 ↑ x3.5<br>• IFNα-inducible protein 27 ↑ x3<br>• TNF (ligand) superfamily member 10 ↑ x2 |
| CELL CYCLE | • Cdk (CDC2-like) ↓ x2<br>• Growth arrest-specific 6 ↑ x1.5<br>• Cdk 5, regulatory subunit 2 (p39) ↑ x1.5<br>• CDC37 (cell division cycle 37, *S. cerevisiae*, homolog) ↑ x1.5<br>• Cdki 1A (p21, Cip 1) ↑ x5 | • Cdki 2D (p19, inhibits CDK4) ↓ x2.5<br>• Cdk like-2 ↓ x2<br>• Cdk 5, regulatory subunit 1 ↑ x2.5<br>• Cdki1A (p21/Cip1) ↑ x6<br>• Cdk like-2 ↓ x2 | • Cyclin I ↓ x1.5<br>• S-phase kinase-associated protein 1A (p19A) ↑ x2<br>• Cdk 6 ↑ x3<br>• Cdk 5, regulatory subunit 1 (p35) ↑ x2<br>• Cyclin D2 ↑ x1.5<br>• S-phase kinase-associated protein 1A ↑ x2<br>• Cdk 2 ↑ x1.5<br>• Follistatin-like 1 ↑ x1.5 | • Cdc-like 5 (cholinesterase-related cell division controller) ↓ x2<br>• Cdk (CDC2-like) ↓ x9<br>• Cdk 5, regulatory subunit 1 (p35) ↓ x5<br>• Growth arrest-specific 1 ↓ x2.5<br>• Cyclin B2 ↓ x2.5 |

TABLE 20
SOM230 Gene Expression Profiling (Continued)

| CLASS | KIDNEY | LIVER | SPLEEN | THYROID |
|---|---|---|---|---|
| APOPTOSIS | • Death effector domain-containing ↓ x3.5<br>• Fas/Apo-1/CD95 ↑ x1.5 | • BCL2-like 1 ↓ x3.5<br>• Rb 1 (including osteosarcoma) ↓ x2<br>• Death-associated protein 6 ↓ x2.5<br>• Rb binding protein ↑ x2.5<br>• Rb-like 2 (p130)↑ x3<br>• Fas-activated serine/threonine kinase ↑ x1.5 | • Rb binding protein ↑ x2<br>• Caspase 8, apoptosis-related cysteine protease ↑ x2.5<br>• TNF (cachectin) ↑ x2<br>• TNF (ligand) superfamily, member 13 ↑ x2.5<br>• Bcl-2 binding component 3 ↑ x2.5<br>• Death-associated protein ↑ x1.5<br>• Tumor protein p53-binding protein ↑ x1.5<br>• Rb-binding protein 8 ↑ x1.5<br>• Programmed cell death 10 ↑ x1.5 | • Bcl-2 binding component 3 ↓ x2 |

[00137] These results show that several signal transduction pathways were affected. They included the phosphatidyl inositol/PKC/phospholipases/calcium-calcineurin-calmodulin pathway, the Ras/MAPK kinase/ERK kinase dependent pathway, the JAK/STAT pathway, and adenylate/guanylate cyclases with their dependent pathways. The changes for the cell surfaces receptors included numerous G-protein coupled receptors, receptors for growth factors and glutamate receptors. The changes in ATP-dependent transport proteins involved ion channels and associated proteins. The compound also affected neuromediators/neuromodulators, pancreatic and gastrointestinal secretions, hormones, cytoskeletal proteins and enzymes/catalysts.

[00138] Examples of genes reflecting several SSTR signaling pathways in the pituitary are shown in TABLE 21. Selected genes from the primary gene lists were produced by a succession of filtering and statistical algorithms (t-test: p value: 0.05). The numerical values correspond to the AvgDiff (see above) of the relevant probe set for each assay with the range of observed values between brackets. Of particular interest in this analysis were the transcript level changes for molecules known to be closely associated with the binding of the natural peptides, SST-14 and SST-28, to the SSTRs.

TABLE 21

Examples of Genes Reflecting Several SSTR Signaling Pathways in the Pituitary

| GENES | CONTROL | SOM230 (0.1 mg/animal/14 day) |
|---|---|---|
| SIGNAL TRANSDUCTION | | |
| 1) Phophatidyl inositol and related pathways/PKC, phospholipases | | |
| • IP-4-phosphatase, type 1, isoform b | 296 (241 to 342) | 177 (107 to 232) |
| • PI-3-kinase, catalytic, δ polypeptide | 91 (45 to 146) | 34 (20 to 67) |
| • PI-3-kinase,catalytic, α polypeptide | 72 (26 to 135) | 21 (20 to 24) |
| • PI transfer protein, β | 125 (93 to 187) | 42 (34 to 50) |
| • PKC inhibitor | 2,351 (2,135 to 2,755) | 3,333 (2,339 to 3,878) |
| • PLC, γ 1 (formerly subtype 148) | 111 (100 to 131) | 40 (20 to 63) |
| • PKC inhibitor | 2,351 (2,1345 to 2,755) | 3,332 (2,339 to 3,878) |
| 2) Ras/MAPK kinase/ERK kinase related pathways and adaptor proteins | | |
| • MAPKKK5 | 171 (148 to 207) | 278 (221 to 351) |
| • Rab geranylgeranyltransferase, α subunit | 164 (152 to 173) | 104 (70 to 172) |
| • Rab geranygeranyltransferase, βsubunit | 230 (187 to 250) | 284 (246 to 374) |
| • SHB adaptor protein (a Src homology 2 protein) | 112 (43 to 190) | 38 (20 to 55) |
| • RAB 5C, member RAS oncogene family | 72 (20 to 138) | 162 (109 to 212) |
| 3) Protein tyrosine phosphatases/other phosphatases | | |
| • Dual specificity phosphatase 8 | 493 (344 to 625) | 170 (67 to 238) |
| • Phosphatase and tensin homolog (mutated in multiple advanced cancers 1) | 129 (58 to 228) | 36 (20 to 63) |
| • PTP, receptor type, T | 58 (41 to 78) | 101 (48 to 129) |
| • PP 1, regulatory (inhibitor) subunit 5 | 20 | 75 (60 to 90) |
| 4) Adenylate/guanylate cyclases and related pathways | | |
| • Soluble adenylyl cyclase | 54 (51 to 57) | 22 (20 to 27) |
| CELL SURFACE RECEPTORS | | |
| 1) G-protein coupled receptors | | |
| • SSTR3 | 22 (20 to 24) | 57 (20 to 90) |
| 2) Glutamate receptor and related binding proteins | | |
| • GLUR 2, precursor | 42 (20 to 86) | 59 (20 to 177) |

TABLE 21

Examples of Genes Reflecting Several SSTR Signaling Pathways in the Pituitary

| GENES | CONTROL | SOM230 (0.1 mg/animal/14 day) |
|---|---|---|
| ATP-DEPENDENT TRANSPORT PROTEINS | | |
| Ion channels and related pathways | | |
| ATPase, Na+/K+ transporting, β 3 polypeptide | 292 (246 to 353) | 610 (335 to 949) |
| ATPase, Na+/K+ transporting, α 2 (+) polypeptide | 86 (52 to 130) | 184 (69 to 325) |
| ATPase, H+/K+ exchanging, α polypeptide | 128 (50 to 245) | 20 |
| K+ channel, subfamily K, member 3 (TASK) | 132 (69 to 188) | 26 (20 to 43) |
| K+ voltage-gated channel, Shab-related subfamily, member 1 | 66 (20 to 112) | 22 (20 to 31) |
| Putative Ca++ transporting ATPase | 61 (38 to 98) | 101 (84 to 112) |
| CELL CYCLE | | |
| Core-binding factor, runt domain, α subunit 2; translocated to, 1; cyclin D-related | 225 (113 to 343) | 491 (251 to 677) |
| Forkhead box O3A | 497 (447 to 553) | 257 (186 to 324) |
| Forkhead box H1 | 225 (113 to 343) | 117 (251 to 677) |
| Cyclin F | 198 (171 to 229) | 74 (48 to 132) |
| Cyclin D3 | 187 (173 to 201) | 338 (202 to 446) |
| S-phase response (cyclin-related) | 91 (88 to 97) | 129 (111 to 148) |
| Cell division cycle 25B | 40 (20 to 67) | 162 (134 to 187) |
| Cdk inhibitor 2C (p18, inhibits CDK4) | 81 (58 to 99) | 184 (140 to 229) |
| Cdk inhibitor 2D (p19, inhibits CDK4) | 198 (171 to 229) | 99 (83 to 118) |
| APOPTOSIS | | |
| BCL2-associated athanogene | 216 (207 to 231) | 318 (235 to 409) |
| BCL2-antagonist of cell death | 44 (33 to 47) | 69 (42 to 89) |
| Bax gamma | 258 (207 to 297) | 326 (221 to 448) |
| BCL2/adenovirus E1B 19kD-interacting protein 3 | 342 (288 to 401) | 458 (388 to 526) |
| Programmed cell death 6 | 504 (443 to 547) | 635 (513 to 747) |
| Neuroblastoma-amplified protein | 178 (149 to 210) | 237 (201 to 258) |

[00139] The effects on the growth hormone/ insulin-like growth factor-1 (GH/IGF-1) and glucagon/insulin axes (Macaulay VM, *Br J Cancer* 65: 311-20 (1992); Pollak MN & Schally AV, *Proc Soc Exp Biol Med* 217: 143-52 (1998)) were reflected in transcript level changes in several organs. The results are shown in TABLE 22. Beside the expected change in IGF-1 transcript level, there was an effect on insulin-like growth factor-2 (IGF-2) as well (in the pituitary and kidneys) that might be useful as a biological marker of SOM230 activity if reflected in the blood. The genes were selected as above in TABLE 21.

TABLE 22
Genes Reflecting the Effects of SOM230
on the GH/IGF and Glucagon/Insulin Axes in Different Tissues

| ORGANS/GENES | CONTROL | SOM230 (0.1mg/animal/14day) |
|---|---|---|
| PITUITARY | | |
| IGF-2 | 126 (40 to 179) | 70 (20 to 150) |
| GR | 20 | 109 (51 to 215) |
| IGFBP, acid labile subunit | 30 (20 to 49) | 83 (20 to 110) |
| SSTR3 | 22 (20 to 24) | 57 (20 to 90) |
| BROWN FAT | | |
| IGF-1 | 548 (279 to 810) | 389 (315 to 449) |
| IGFBP 4 | 1410 (916 to 2173) | 763 (429 to 1058) |
| IRS 2 | 48 (20 to 84) | 146 (80 to 222) |
| SSTR3 | 25 (20 to 52) | 194 (87 to 248) |
| PANCREAS | | |
| IGF-1 | 20 | 89 (20 to 298) |
| SSTR2 | 258 (205 to 366) | 156 (120 to 210) |
| KIDNEY | | |
| IR | 654 (187 to 1,187) | 196 (163 to 265) |
| IGF-2 | 117 (47 to 176) | 49 (20 to 39) |
| IGF-1 | 65 (24 to 103) | 25 (20 to 39) |
| IGFBP2 | 375 (211 to 625) | 563 (457 to 655) |
| SSTR 3 | 31 (20 to 69) | 82 (33 to 120) |
| SSTR 2 | 74 (20 to 153) | 126 (93 to 158) |
| LIVER | | |
| Insulin promoter factor 1, homeodomain transcription factor | 89 (58 to 160) | 42 (23 to 52) |
| IGF-2 | 701 (403 to 961) | 269 (224 to 291) |
| IGFBP2 | 2,722 (1,321 to 3,363) | 4,476 (3,191 to 5,422) |
| GR | 44 (20 to 82) | 80 (70 to 360) |
| SPLEEN | | |
| IGFBP6 | 495 (130 to 982) | 1,043 (853 to 1,155) |
| IGF-1 | 72 (42 to 103) | 85 (52 to 125) |
| SSTR2 | 56 (20 to 83) | 93 (87 to 95) |
| THYROID | | |
| IGF-1 | 91 (20 to 179) | 58 (20 to 114) |

[00140] Other genes of interest affected by SOM230 were the transcript levels of growth factors (PDGF, FGF, EGF, TGFβ), their receptors and factors of angiogenesis (PDGF, VEGF, thrombospondin) involved in tumor growth and spreading (Woltering EA *et al., New Drugs* 15: 77-86 (1997)). Also reported for somatostatin and analogues, genes involved in immunity were changed, *i.e.* cytokines (IL-1, TNF, IFN), regulators of T and B cell genesis and function (CD2 antigen, IL-2 receptor, B-lymphoid tyrosine kinase, IL-2 inducible T cell kinase, p56lck, RAG1, TCRζ chain precursor, RAG2, FLT 3 ligand) (van Hagen PM *et al. Eur J Clin Invest* 24: 91-9 (1994)), as well as genes involved in blood pressure control and diuresis, *i.e.* atrial natriuretic peptide and its receptor guanylyl cyclase A, arginine vasopressin and its receptor (Aguilera G *et al., Nature* 292: 262-3 (1981); Aguilera G *et al., Endocrinology* 111: 1376-84 (1982); Ray C *et al., Clin Sci (Lond)* 84: 455-60 (1993); Cheng

H *et al., Biochem J* 364: 33-9 (2002)). A specific gene involved in the control of fat storage is the adrenergic β3 receptor in brown fat (Bachman E *et al., Science* 297: 843- 45 (2002)).

[00141] Protein products of the above genes are useful as surrogate markers of the biological activity of SOM230, especially the findings for IGF-2 in the pituitary and kidneys.

[00142] To conclude, the gene profiling of monkey tissues treated with SOM230 at sub-therapeutic is a sensitive approach to identify signaling and effector pathways known for somatostatin. The finding of clear transcriptional signatures for this agonist argues for a comparison with gene expression changes induced by Sandostatin®.

EXAMPLE

SALMON CALCITONIN AND PTS893, PHARMACOGENOMICS EXPLORATORY STUDY IN MONKEYS; MICROARRAY GENE EXPRESSION ANALYSIS

[00143] Calcitonins are endogenous regulator of calcium homeostasis and can be used as anti resorptive agents for the treatment of hypercalcaemia-associated disorders. Various calcitonins, including *e.g.* salmon and eel calcitonin, are commercially available and are commonly employed in the treatment of *e.g.* Paget's disease and osteoporosis. *See*, U.S. Pat. Nos. 5,733,569 and 5,759,565, the contents of which are incorporated by reference. See also, U.S. Pat. Nos. 5,719,122, 5,175,146, and 5,698,6721, and U.S. Pat. Appln. 003015815. A version of calcitonin (Miacalcin®) is available as a nasal spray. Information regarding the administration of Miacalcin® (calcitonin-salmon) nasal spray is available in the Miacalcin® Prescribing Information (Novartis, November 2002).

[00144] Parathyroid hormone (PTH) is a polypeptide of 84 amino acids. Parathyroid hormone regulates bone remodeling and $Ca^{2+}$ homeostasis. Parathyroid hormone is also a known paracrine activator of osteoclast differentiation and activity. PTS893 is an analogue of the endogenous parathyroid hormone, in which certain sites of chemical instability are eliminated within N-terminal parathyroid hormone fragments by making appropriate amino acid substitutions at particular residues which results in stable and biologically active human parathyroid hormone fragments. PTS893 [SDZ PTS 893; Leu8, Asp10, Lys11, Ala16, Gln18, Thr33, Ala34 human PTH 1-34 [hPTH(1-34)]] is a 34 amino acid parathyroid analogue that enhances bone mass and biomechanical properties. Kneissel M *et al., Bone* 28: 237-50 (March 2001); Stewart AF *et al., J. Bone. Miner. Res.* 15(8): 1517-25 (August 2000); Thomsen JS *et al., Bone* 25(5):561-9 (November 1999). N-terminal fragments of human parathyroid hormones include hPTH(1-34)OH muteins and hPTH(1-38)OH muteins. PTS893 comprises at

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SALMON CALCITONIN AND PTS893, PHARMACOGENOMICS EXPLORATORY STUDY IN MONKEYS; MICROARRAY GENE EXPRESSION ANALYSIS

[00143] Calcitonins are endogenous regulator of calcium homeostasis and can be used as anti resorptive agents for the treatment of hypercalcaemia-associated disorders. Various calcitonins, including *e.g.* salmon and eel calcitonin, are commercially available and are commonly employed in the treatment of *e.g.* Paget's disease and osteoporosis. *See*, U.S. Pat. Nos. 5,733,569 and 5,759,565, the contents of which are incorporated by reference. See also, U.S. Pat. Nos. 5,719,122, 5,175,146, and 5,698,6721, and U.S. Pat. Appln. 003015815. A version of calcitonin (Miacalcin®) is available as a nasal spray. Information regarding the administration of Miacalcin® (calcitonin-salmon) nasal spray is available in the Miacalcin® Prescribing Information (Novartis, November 2002).

[00144] Parathyroid hormone (PTH) is a polypeptide of 84 amino acids. Parathyroid hormone regulates bone remodeling and $Ca^{2+}$ homeostasis. Parathyroid hormone is also a known paracrine activator of osteoclast differentiation and activity. PTS893 is an analogue of the endogenous parathyroid hormone, in which certain sites of chemical instability are eliminated within N-terminal parathyroid hormone fragments by making appropriate amino acid substitutions at particular residues which results in stable and biologically active human parathyroid hormone fragments. PTS893 [SDZ PTS 893; Leu8, Asp10, Lys11, Ala16, Gln18, Thr33, Ala34 human PTH 1-34 [hPTH(1-34)]] is a 34 amino acid parathyroid analogue that enhances bone mass and biomechanical properties. Kneissel M *et al.*, *Bone* 28: 237-50 (March 2001); Stewart AF *et al.*, *J. Bone. Miner. Res.* 15(8): 1517-25 (August 2000); Thomsen JS *et al.*, *Bone* 25(5):561-9 (November 1999). N-terminal fragments of human parathyroid hormones include hPTH(1-34)OH muteins and hPTH(1-38)OH muteins. PTS893 comprises at

least the first 27 N-terminal amino acid units of parathyroid hormone. Preferred parathyroid hormone derivatives are those comprising at least one amino acid unit replaced in one or more of the following positions of the parathyroid hormone sequence: 8-11, 13, 16-19, 21, 22, 29 to 34, particularly 8-11, 16-19, 33 and/or 34. These compounds exhibit desirable bone-forming properties both *in vivo* and *in vitro* which are equal to or above the level of natural PTH and its N-terminal fragments. See, European patent EP 0 672 057; published PCT patent application WO 94/02510; Kneissel M *et al., Bone* 28: 237-50 (March 2001); Stewart AF *et al., J Bone Miner Res* 15(8): 1517-25 (August 2000); Thomsen JS *et al., Bone* 25(5):561-9 (November 1999).

[00145] *Introduction and summary.* The purpose of this EXAMPLE was to evaluate the gene expression changes in cynomolgus monkeys following a two-week subcutaneous treatment with salmon calcitonin (sCT) at 50 μg/animal/day and PTS893 at 5 μg/animal/day to elucidate the mechanisms of action mediating their effects as well as the identification of biomarkers of therapeutic indications. This EXAMPLE is believed to be the first analysis that globally describes the molecular mechanisms of action of salmon calcitonin and a parathyroid hormone analogue by multiorgan-gene-profiling analysis in primates. This is also believed to be the first gene profiling analysis which describes the molecular mechanisms of action of hormonal-mediated bone remodeling by salmon calcitonin and PTS893.

[00146] In this EXAMPLE, salmon calcitonin and PTS893 were both found to have modulating effects on genes affecting the direct, autocrine, paracrine and endocrine regulation of the mesenchymal cell functions such as transforming growth factor betas (TGF-βs), insulin-like growth factors (IGFs), bone morphogenetic proteins (BMPs) and vascular endothelial growth factor (VEGF). Both compounds also regulate the synthesis and degradation of extracellular matrix components. Salmon calcitonin also regulates estrogen receptor and steroidogenic factor, whereas PTS893 produced a strong up-regulation on nuclear receptors of the steroid/thyroid receptor family. These data therefore support the role of calcitonin as an anabolic agent.

[00147] In addition, salmon calcitonin and PTS893 also influenced some aspects of the mineralization of the extracellular matrix, since changes in amelogenin, dentin and ectonucleotide pyrophosphatases were observed.

[00148] In addition, PTS893 showed an effect on mediating the paracrine activation of osteoclast differentiation and activity, through cytokine and RANK ligand.

[00149] No significant differences in gene expression profiling were attributable to the fact of administering salmon calcitonin and PTS893 in combination, with respect to the single therapy.

[00150] Thus, gene profiling analysis in this EXAMPLE allowed the reconstruction of the pathways involved in calcitonin and parathyroid hormone signal transduction, triggered by protein-G-linked-receptor stimulation and their influence on cell cycle, as indicated by the changes observed in cyclins.

[00151] *Animals*. A two-week subcutaneous treatment was carried out with salmon calcitonin (sCT), PTS893 or a combination of the two, each of which were dissolved in phosphate buffered saline (PBS) containing 9% autologous serum. Solvent was used as vehicle for the control group.

[00152] The animals used in this analysis were cynomolgus monkeys (*Macaca fascicularis*), supplied by Centre de Recherches Primatologiques, Port Louis, Mauritius. Two animals were used per group and sex. At the beginning of the treatment period, the animals were at least 24 months old, with a body weight of approximately 3 kg. Animals were kept under standard conditions for animal welfare. Animals were examined daily for mortality, food consumption and clinical observations. Body weight was recorded once per week. The dosages were 0 μg/animal/day (as the control), 50 μg/animal/day of salmon calcitonin and 5 μg/animal/day of PTS893.

[00153] *In vivo examinations.* No significant histopathological changes were observed. No relevant changes were observed other than a body weight decrease ranging from 8 to 12% in the salmon calcitonin group. A decrease in food consumption was also observed, although not always consistent with the decrease in body weight.

TABLE 23
Food Consumption – Males

Control

| Day | -6 | -5 | -4 | -3 | -2 | -1 | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Animal no. W62501 | 50 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Animal no. W62502 | 50 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 25 |
| Day | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | | Avg. |
| Animal no. W62501 | 75 | 100 | 100 | 100 | 100 | 100 | 100 | 25 | | | 91.7 |
| Animal no. W62502 | 100 | 75 | 75 | 100 | 100 | 100 | 75 | 100 | 50 | | 91.7 |
| Both animals | | | | | | | | | | | 91.7 |

Salmon Calcitonin

| Day | -6 | -5 | -4 | -3 | -2 | -1 | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Animal no. W62503 | 50 | 75 | 50 | 75 | 100 | 75 | 75 | 25 | 50 | 100 | 100 |
| Animal no. W62504 | 50 | 75 | 75 | 75 | 100 | 75 | 50 | 25 | 100 | 75 | 100 |
| Day | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | | Avg. |
| Animal no.W62503 | 75 | 100 | 100 | 100 | 100 | 75 | 75 | 25 | | | 70.8 |
| Animal no.W62504 | 75 | 75 | 75 | 100 | 100 | 75 | 75 | 25 | 75 | | 75.0 |
| Both animals | | | | | | | | | | | 72.9 |

PTS893

| Day | -6 | -5 | -4 | -3 | -2 | -1 | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Animal no. W62505 | 50 | 100 | 100 | 100 | 75 | 100 | 100 | 100 | 100 | 100 | 100 |
| Animal no. W62506 | 50 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Day | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | | Avg. |
| Animal no. W62505 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 50 | 75 | | 87.5 |
| Animal no. W62506 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | | 91.7 |
| Both animals | | | | | | | | | | | 89.6 |

[00154] The animals to whom salmon calcitonin was administered presented with a decrease in body weight ranging between 8 to 12%, which can be attributed to a decrease in food consumption. An anorectic effect had previously been described for salmon calcitonin acting through amylin receptors Eiden S *et al., J. Physiol.* 541(pt3): 1041-1048 (2002); Lutz TA *et al., Peptides* 21 (2): 233-8 (2000). However, no signs of toxicity were observed here. Hormonal and lipid changes observed in this EXAMPLE are most probably related to a consequent metabolic adaptation.

[00155] No relevant changes in electrocardiograms (ECG) or blood pressure were observed.

TABLE 24
Blood Pressure

| Animal number | Sex | Compound administered | Week -1 (mm Hg) | Week 2 (mm Hg) | Difference (mm Hg) |
|---|---|---|---|---|---|
| W62501 | Male | Control | 121 | 98 | -23 |
| W62501 | Male | Control | 90 | 29 | -61 |
| W62502 | Male | Control | 86 | 107 | 21 |
| W62502 | Male | Control | 26 | 34 | 8 |
| W62503 | Male | Salmon Calcitonin | 135 | 99 | -36 |
| W62503 | Male | Salmon Calcitonin | 61 | 40 | -21 |
| W62504 | Male | Salmon Calcitonin | 102 | 79 | -23 |
| W62504 | Male | Salmon Calcitonin | 56 | 35 | -21 |
| W62505 | Male | PTS893 | 76 | 87 | 11 |
| W62505 | Male | PTS893 | 18 | 22 | 4 |
| W62506 | Male | PTS893 | 106 | 101 | -5 |
| W62506 | Male | PTS893 | 53 | 33 | -20 |
| W62551 | Female | Control | 96 | 76 | -20 |
| W62551 | Female | Control | 27 | 26 | -1 |
| W62552 | Female | Control | 102 | 93 | -9 |
| W62552 | Female | Control | 26 | 36 | 10 |
| W62553 | Female | Salmon Calcitonin | 98 | 82 | -16 |
| W62553 | Female | Salmon Calcitonin | 50 | 25 | -25 |
| W62554 | Female | Salmon Calcitonin | 92 | 44 | -48 |
| W62554 | Female | Salmon Calcitonin | 26 | 30 | 4 |
| W62555 | Female | PTS893 | 92 | 70 | -22 |
| W62555 | Female | PTS893 | 43 | 42 | -1 |
| W62556 | Female | PTS893 | 78 | 87 | 9 |
| W62556 | Female | PTS893 | 24 | 28 | 4 |

[00156] *Blood sampling*. Animals were fasted overnight before blood collection but had free access to water. Blood samples were taken from a peripheral vein. Standard haematology and clinical chemistry analysis were performed once during pretest and at the end of the treatment period. Blood samples were collected from each animal at the same intervals as described for the clinical chemistry investigations. The serum samples were deep-frozen (approximately -80°C) until analyses for hormone determination.

[00157] *Clinical chemistry and hormone determinations.* A slight anaemia was observed in all animals of the study, including the controls. This was attributed to the repeated blood sampling and not considered to be relevant.

TABLE 25
Hematology – Males

Control

| Animal no. | | | W62501 | | | W62502 | |
|---|---|---|---|---|---|---|---|
| Test | Units | d-6 | d7 | d13 | d-6 | d7 | d13 |
| WBC | G/l | 10.0 | 11.1 | 12.9 | 6.1 | 11.2 | 6.3 |
| RBC | T/l | 7.3 | 6.5 | 6.4 | 6.8 | 6.5 | 6.2 |
| HB | g/dl | 12.9 | 11.9 | 11.7 | 13.1 | 12.3 | 11.9 |
| PCV | l/l | 0.44 | 0.40 | 0.44 | 0.42 | 0.41 | 0.41 |
| MCV | fl | 60 | 61 | 68 | 61 | 63 | 66 |
| MCH | pg | 17.8 | 18.2 | 18.1 | 19.3 | 19.0 | 19.0 |
| MCHC | g/dl | 29.8 | 29.6 | 26.8 | 31.5 | 30.1 | 28.9 |
| PLAT | G/l | 316 | 371 | 266 | 458 | 500 | 547 |
| N | G/l | 6.46 | 4.93 | 3.65 | 2.09 | 6.77 | 1.24 |
| E | G/l | 0.01 | 0.14 | 0.20 | 0.10 | 0.10 | 0.10 |
| B | G/l | 0.02 | 0.03 | 0.06 | 0.02 | 0.02 | 0.00 |
| L | G/l | 3.05 | 5.45 | 8.44 | 3.60 | 3.65 | 4.51 |
| M | G/l | 0.46 | 0.51 | 0.54 | 0.33 | 0.64 | 0.46 |

Salmon Calcitonin

| Animal no. | | | W62503 | | | W62504 | |
|---|---|---|---|---|---|---|---|
| Test | Units | d-6 | d7 | d13 | d-6 | d7 | d13 |
| WBC | G/l | 7.7 | 11.8 | 8.0 | 11.5 | 9.5 | 8.8 |
| RBC | T/l | 6.3 | 5.9 | 5.6 | 6.9 | 6.0 | 5.4 |
| HB | g/dl | 12.6 | 11.7 | 11.2 | 13.6 | 11.5 | 10.3 |
| PCV | l/l | 0.40 | 0.39 | 0.39 | 0.43 | 0.37 | 0.36 |
| MCV | fl | 64 | 66 | 70 | 62 | 62 | 67 |
| MCH | pg | 20.2 | 19.9 | 20.2 | 19.7 | 19.2 | 19.2 |
| MCHC | g/dl | 31.4 | 30.3 | 29.0 | 32.0 | 31.3 | 28.7 |
| PLAT | G/l | 351 | 396 | 302 | 247 | 330 | 389 |
| N | G/l | 3.36 | 4.11 | 1.90 | 3.93 | 3.31 | 3.04 |
| E | G/l | 0.02 | 0.10 | 0.13 | 0.16 | 0.09 | 0.01 |
| B | G/l | 0.02 | 0.04 | 0.03 | 0.08 | 0.04 | 0.03 |
| L | G/l | 4.00 | 6.79 | 5.38 | 6.55 | 5.57 | 4.92 |
| M | G/l | 0.30 | 0.73 | 0.57 | 0.76 | 0.45 | 0.76 |

d-6, d7 and d13 indicate day -6, day 7 and day 13 relative to the starting day of dosing

## TABLE 25
## Hematology – Males

### PTS893

| Animal no. | | | W62505 | | | W62506 | |
|---|---|---|---|---|---|---|---|
| Test | Units | d-6 | d7 | d13 | d-6 | d7 | d13 |
| WBC | G/l | 10.4 | 8.4 | 8.8 | 9.1 | 15.0 | 11.9 |
| RBC | T/l | 7.6 | 6.4 | 6.8 | 6.5 | 5.9 | 5.8 |
| HB | g/dl | 13.6 | 11.3 | 11.7 | 13.2 | 11.9 | 11.8 |
| PCV | l/l | 0.43 | 0.38 | 0.43 | 0.40 | 0.40 | 0.41 |
| MCV | fl | 57 | 60 | 63 | 62 | 67 | 70 |
| MCH | pg | 18.0 | 17.7 | 17.3 | 20.4 | 20.2 | 20.3 |
| MCHC | g/dl | 31.5 | 29.3 | 27.5 | 33.1 | 30.2 | 29.2 |
| PLAT | G/l | 325 | 456 | 330 | 459 | 589 | 452 |
| N | G/l | 4.45 | 1.77 | 2.88 | 4.80 | 8.73 | 6.51 |
| E | G/l | 0.21 | 0.30 | 0.19 | 0.03 | 0.08 | 0.07 |
| B | G/l | 0.00 | 0.02 | 0.04 | 0.02 | 0.03 | 0.03 |
| L | G/l | 5.07 | 5.91 | 5.37 | 3.99 | 5.30 | 4.86 |
| M | G/l | 0.62 | 0.39 | 0.27 | 0.27 | 0.83 | 0.46 |

d-6, d7 and d13 indicate day -6, day 7 and day 13 relative to the starting day of dosing

## TABLE 26
## Hematology – Females

### Control

| Animal no. | | | W62551 | | | W62552 | |
|---|---|---|---|---|---|---|---|
| Test | Units | d-8 | d7 | d13 | d-8 | d7 | d13 |
| WBC | pg/ml | 8.2 | 13.7 | 10.0 | 10.1 | 9.1 | 10.4 |
| RBC | nmol/l | 6.5 | 6.2 | 5.8 | 6.7 | 6.2 | 5.8 |
| HB | pg/ml | 12.8 | 11.8 | 11.3 | 13.1 | 11.7 | 11.4 |
| PCV | mU/l | 0.42 | 0.43 | 0.41 | 0.42 | 0.42 | 0.41 |
| MCV | pg/ml | 64 | 69 | 71 | 63 | 68 | 70 |
| MCH | ng/ml | 19.7 | 19.1 | 19.4 | 19.5 | 18.9 | 19.5 |
| MCHC | pg/ml | 30.6 | 27.7 | 27.4 | 30.9 | 27.7 | 27.9 |
| PLAT | nmol/l | 463 | 445 | 468 | 286 | 292 | 275 |
| N | nmol/l | 4.45 | 5.86 | 3.53 | 6.69 | 3.13 | 4.23 |
| E | mUI/l | 0.03 | 0.13 | 0.12 | 0.01 | 0.15 | 0.19 |
| B | pg/ml | 0.03 | 0.07 | 0.04 | 0.02 | 0.03 | 0.03 |
| L | pg/ml | 3.40 | 7.09 | 5.91 | 3.14 | 5.39 | 5.34 |
| M | nmol/l | 0.27 | 0.51 | 0.39 | 0.25 | 0.39 | 0.59 |

d-8, d7 and d13 indicate day -8, day 7 and day 13 relative to the starting day of dosing

## TABLE 27
## Hematology – Females

### Salmon Calcitonin

| Animal no. | | | W62553 | | | W62554 | |
|---|---|---|---|---|---|---|---|
| Test | Units | d-8 | d7 | d13 | d-8 | d7 | d13 |
| WBC | pg/ml | 7.0 | 9.5 | 12.0 | 8.3 | 17.0 | 13.3 |
| RBC | nmol/l | 6.5 | 6.2 | 5.2 | 7.0 | 6.6 | 5.7 |
| HB | pg/ml | 12.3 | 11.5 | 10.1 | 13.8 | 12.7 | 11.0 |
| PCV | mU/l | 0.40 | 0.40 | 0.33 | 0.45 | 0.44 | 0.37 |
| MCV | pg/ml | 61 | 64 | 64 | 65 | 68 | 65 |
| MCH | ng/ml | 19.1 | 18.6 | 19.5 | 19.8 | 19.4 | 19.5 |
| MCHC | pg/ml | 31.2 | 29.0 | 30.3 | 30.6 | 28.7 | 29.9 |
| PLAT | nmol/l | 549 | 594 | 451 | 304 | 356 | 229 |
| N | nmol/l | 3.45 | 3.83 | 5.41 | 3.13 | 9.82 | 6.16 |
| E | mUI/l | 0.03 | 0.36 | 0.73 | 0.03 | 0.04 | 0.06 |
| B | pg/ml | 0.02 | 0.03 | 0.03 | 0.01 | 0.07 | 0.05 |
| L | pg/ml | 3.26 | 4.61 | 5.18 | 4.79 | 6.21 | 6.58 |
| M | nmol/l | 0.25 | 0.63 | 0.69 | 0.30 | 0.82 | 0.39 |

### PTS893

| Animal no. | | | W62555 | | | W62556 | |
|---|---|---|---|---|---|---|---|
| Test | Units | d-8 | d7 | d13 | d-8 | d7 | d13 |
| WBC | pg/ml | 10.1 | 18.4 | 13.2 | 14.3 | 12.3 | 10.1 |
| RBC | nmol/l | 6.9 | 6.2 | 5.9 | 6.7 | 6.4 | 5.9 |
| HB | pg/ml | 13.4 | 11.7 | 11.3 | 12.9 | 12.1 | 11.3 |
| PCV | mU/l | 0.44 | 0.41 | 0.40 | 0.43 | 0.43 | 0.39 |
| MCV | pg/ml | 63 | 67 | 67 | 64 | 68 | 66 |
| MCH | ng/ml | 19.3 | 18.9 | 19.3 | 19.3 | 19.0 | 19.2 |
| MCHC | pg/ml | 30.6 | 28.2 | 28.6 | 30.2 | 28.1 | 29.2 |
| PLAT | nmol/l | 501 | 525 | 496 | 213 | 382 | 309 |
| N | nmol/l | 5.34 | 10.8 | 6.36 | 9.05 | 5.49 | 4.18 |
| E | mUI/l | 0.00 | 0.12 | 0.21 | 0.26 | 0.49 | 0.29 |
| B | pg/ml | 0.00 | 0.06 | 0.03 | 0.03 | 0.04 | 0.04 |
| L | pg/ml | 3.92 | 6.29 | 5.81 | 4.40 | 5.87 | 5.21 |
| M | nmol/l | 0.80 | 1.12 | 0.82 | 0.54 | 0.44 | 0.37 |

d-8, d7 and d13 indicate day -8, day 7 and day 13 relative to the starting day of dosing

[00158] Among the standard clinical chemistry tests performed, slight to moderate decreases in phosphorus and/or magnesium and a moderate to marked decrease in triglycerides were seen in the groups administered salmon calcitonin and PTS893.

TABLE 28
Clinical Chemistry – Males

Control

| Animal no. | | W62501 | | | W62502 | | |
|---|---|---|---|---|---|---|---|
| Test | Units | d-6 | d7 | d13 | d-6 | d7 | d13 |
| Na+ | mmol/l | 154 | 151 | 153 | 152 | 153 | 148 |
| K+ | mmol/l | 4.05 | 5.31 | 4.26 | 4.09 | 4.05 | 4.51 |
| Cl- | mmol/l | 109 | 113 | 108 | 107 | 110 | 111 |
| Ca++ | mmol/l | 2.57 | 2.47 | 2.69 | 2.72 | 2.52 | 2.75 |
| I.PHOS | mmol/l | 2.21 | 1.93 | 2.76 | 1.88 | 1.69 | 1.99 |
| Mg++ | mmol/l | 1.09 | 0.91 | 0.95 | 0.88 | 0.79 | 1.14 |
| GLUC | mmol/l | 3.85 | 4.51 | 4.68 | 3.44 | 5.30 | 6.13 |
| UREA | mmol/l | 9.7 | 4.9 | 5.0 | 7.6 | 6.1 | 5.2 |
| CREAT | µmol/l | 85 | 60 | 75 | 65 | 55 | 57 |
| TOT.BIL. | µmol/l | 6.0 | 2.0 | 2.0 | 7.0 | 3.0 | 4.0 |
| PROT | g/l | 89 | 80 | 88 | 90 | 83 | 85 |
| A/G | | 1.89 | 1.57 | 1.45 | 1.62 | 1.53 | 1.50 |
| CHOL | mmol/l | 3.30 | 3.20 | 3.50 | 3.30 | 3.40 | 3.10 |
| HDL-CHOL | mmol/l | 1.49 | 1.45 | 1.70 | 1.54 | 1.45 | 1.49 |
| LDL-CHOL | mmol/l | 1.63 | 1.62 | 1.84 | 1.56 | 1.93 | 1.49 |
| TRIG | mmol/l | 0.94 | 0.36 | 0.43 | 0.65 | 0.36 | 0.45 |
| ALP | IU/l | 1559 | 1241 | 1313 | 1463 | 1423 | 1493 |
| BAP-E | IU/l | 543 | 439 | 457 | 452 | 476 | 464 |
| ASAT | IU/l | 22 | 22 | 25 | 30 | 26 | 26 |
| ALAT | IU/l | 22 | 32 | 30 | 29 | 41 | 37 |
| CK | IU/l | 150 | 45 | 127 | 74 | 67 | 102 |
| LDH | IU/l | 392 | 585 | 549 | 421 | 518 | 592 |
| GGT | IU/l | 128 | 92 | 111 | 89 | 71 | 75 |
| ALB | % | 65 | 61 | 59 | 62 | 61 | 60 |
| A1-GLOB | % | 1.90 | 2.70 | 2.50 | 1.90 | 2.10 | 2.30 |
| A2-GLOB | % | 7.60 | 8.30 | 7.90 | 8.20 | 8.90 | 8.50 |
| B-GLOB | % | 16 | 18 | 19 | 18 | 19 | 19 |
| G-GLOB | % | 9.2 | 9.9 | 10.9 | 9.6 | 9.3 | 10.2 |
| ALB | g/l | 58 | 49 | 52 | 56 | 50 | 51 |
| A1-GLOB | g/l | 1.70 | 2.20 | 2.20 | 1.70 | 1.70 | 2.00 |
| A2-GLOB | g/l | 6.80 | 6.60 | 7.00 | 7.40 | 7.40 | 7.20 |
| B-GLOB | g/l | 14 | 14 | 17 | 17 | 16 | 16 |
| G-GLOB | g/l | 8.2 | 7.9 | 9.6 | 8.6 | 7.7 | 8.7 |

d-6, d7 and d13 indicate day -6, day 7 and day 13 relative to the starting day of dosing

## TABLE 28
## Clinical Chemistry – Males

### Salmon Calcitonin

| Animal no. | | | W62503 | | | W62504 | |
|---|---|---|---|---|---|---|---|
| Test | Units | d-6 | d7 | d13 | d-6 | d7 | d13 |
| Na+ | mmol/l | 151 | 145 | 148 | 154 | 142 | 144 |
| K+ | mmol/l | 4.24 | 4.90 | 4.34 | 4.85 | 5.15 | 4.48 |
| Cl- | mmol/l | 107 | 104 | 104 | 113 | 106 | 101 |
| Ca++ | mmol/l | 2.66 | 2.68 | 2.91 | 2.71 | 2.54 | 2.73 |
| I.PHOS | mmol/l | 2.05 | 1.67 | 2.06 | 2.10 | 1.73 | 1.94 |
| Mg++ | mmol/l | 0.97 | 0.68 | 0.73 | 0.99 | 0.71 | 0.72 |
| GLUC | mmol/l | 3.57 | 3.58 | 4.29 | 3.70 | 4.98 | 6.19 |
| UREA | mmol/l | 7.9 | 1.3 | 2.9 | 6.6 | 3.3 | 2.9 |
| CREAT | µmol/l | 78 | 57 | 62 | 64 | 50 | 56 |
| TOT.BIL. | µmol/l | 5.0 | 2.0 | 1.0 | 3.0 | 2.0 | 2.0 |
| PROT | g/l | 87 | 82 | 87 | 91 | 83 | 89 |
| A/G | | 1.76 | 1.68 | 1.42 | 1.42 | 1.26 | 1.05 |
| CHOL | mmol/l | 3.30 | 3.60 | 3.70 | 3.80 | 3.90 | 3.40 |
| HDL-CHOL | mmol/l | 1.49 | 2.09 | 2.44 | 1.46 | 1.48 | 1.39 |
| LDL-CHOL | mmol/l | 1.21 | 1.28 | 1.26 | 1.87 | 2.51 | 1.83 |
| TRIG | mmol/l | 0.96 | 0.24 | 0.27 | 0.92 | 0.22 | 0.68 |
| ALP | IU/l | 1488 | 1023 | 1226 | 857 | 587 | 626 |
| BAP-E | IU/l | 508 | 363 | 302 | 311 | 188 | 180 |
| ASAT | IU/l | 28 | 31 | 28 | 24 | 17 | 24 |
| ALAT | IU/l | 38 | 39 | 43 | 48 | 24 | 31 |
| CK | IU/l | 124 | 56 | 119 | 75 | 45 | 173 |
| LDH | IU/l | 439 | 400 | 427 | 356 | 384 | 519 |
| GGT | IU/l | 105 | 80 | 75 | 121 | 75 | 69 |
| ALB | % | 64 | 63 | 59 | 59 | 56 | 51 |
| A1-GLOB | % | 1.60 | 2.00 | 2.40 | 1.90 | 2.80 | 3.60 |
| A2-GLOB | % | 8.00 | 8.80 | 8.80 | 8.70 | 8.70 | 7.80 |
| B-GLOB | % | 18 | 18 | 20 | 19 | 21 | 24 |
| G-GLOB | % | 8.3 | 8.5 | 9.7 | 12.0 | 12.1 | 13.6 |
| ALB | g/l | 56 | 51 | 51 | 54 | 46 | 46 |
| A1-GLOB | g/l | 1.40 | 1.60 | 2.10 | 1.70 | 2.30 | 3.20 |
| A2-GLOB | g/l | 7.00 | 7.20 | 7.70 | 7.90 | 7.20 | 6.90 |
| B-GLOB | g/l | 16 | 15 | 18 | 17 | 17 | 21 |
| G-GLOB | g/l | 7.2 | 7.0 | 8.4 | 10.9 | 10.0 | 12.1 |

d-6, d7 and d13 indicate day -6, day 7 and day 13 relative to the starting day of dosing

## TABLE 28
## Clinical Chemistry – Males

## PTS893

| Animal no. | | | W62505 | | | W62506 | |
|---|---|---|---|---|---|---|---|
| Test | Units | d-6 | d7 | d13 | d-6 | d7 | d13 |
| Na+ | mmol/l | 151 | 151 | 152 | 151 | 149 | 149 |
| K+ | mmol/l | 5.13 | 4.00 | 4.27 | 4.72 | 4.76 | 4.12 |
| Cl- | mmol/l | 110 | 107 | 110 | 112 | 106 | 106 |
| Ca++ | mmol/l | 2.81 | 2.39 | 2.59 | 2.64 | 2.45 | 2.51 |
| I.PHOS | mmol/l | 2.59 | 1.68 | 2.22 | 2.12 | 1.12 | 1.77 |
| Mg++ | mmol/l | 1.04 | 0.71 | 0.77 | 0.97 | 0.70 | 0.76 |
| GLUC | mmol/l | 5.09 | 4.76 | 5.42 | 3.88 | 5.26 | 4.96 |
| UREA | mmol/l | 11.6 | 3.7 | 6.4 | 15.0 | 4.9 | 5.8 |
| CREAT | µmol/l | 86 | 66 | 79 | 77 | 63 | 70 |
| TOT.BIL. | µmol/l | 5.0 | 2.0 | 1.0 | 7.0 | 2.0 | 1.0 |
| PROT | g/l | 81 | 74 | 81 | 88 | 86 | 89 |
| A/G | | 1.89 | 1.70 | 1.76 | 1.58 | 1.28 | 1.40 |
| CHOL | mmol/l | 3.20 | 3.30 | 3.10 | 2.50 | 2.50 | 2.60 |
| HDL-CHOL | mmol/l | 1.49 | 1.49 | 1.61 | 1.24 | 1.25 | 1.38 |
| LDL-CHOL | mmol/l | 1.39 | 1.73 | 1.51 | 1.27 | 1.22 | 1.38 |
| TRIG | mmol/l | 0.96 | 0.30 | 0.63 | 0.49 | 0.39 | 0.35 |
| ALP | IU/l | 1703 | 1494 | 1768 | 1414 | 1363 | 1486 |
| BAP-E | IU/l | 523 | 532 | 564 | 445 | 423 | 497 |
| ASAT | IU/l | 24 | 18 | 24 | 25 | 27 | 29 |
| ALAT | IU/l | 32 | 30 | 27 | 23 | 19 | 20 |
| CK | IU/l | 111 | 82 | 148 | 86 | 73 | 125 |
| LDH | IU/l | 367 | 400 | 528 | 354 | 432 | 464 |
| GGT | IU/l | 133 | 99 | 105 | 112 | 85 | 91 |
| ALB | % | 66 | 63 | 64 | 61 | 56 | 59 |
| A1-GLOB | % | 2.20 | 2.80 | 2.60 | 2.40 | 3.60 | 2.80 |
| A2-GLOB | % | 8.80 | 8.90 | 8.70 | 7.30 | 8.30 | 7.50 |
| B-GLOB | % | 17 | 18 | 19 | 19 | 22 | 20 |
| G-GLOB | % | 6.9 | 6.9 | 6.3 | 9.8 | 10.5 | 10.9 |
| ALB | g/l | 53 | 47 | 52 | 54 | 48 | 52 |
| A1-GLOB | g/l | 1.80 | 2.10 | 2.10 | 2.10 | 3.10 | 2.50 |
| A2-GLOB | g/l | 7.10 | 6.60 | 7.10 | 6.40 | 7.10 | 6.70 |
| B-GLOB | g/l | 14 | 14 | 15 | 17 | 19 | 18 |
| G-GLOB | g/l | 5.6 | 5.1 | 5.1 | 8.6 | 9.0 | 9.7 |

d-6, d7 and d13 indicate day -6, day 7 and day 13 relative to the starting day of dosing

## TABLE 29
## Clinical Chemistry – Females

### Control

| Animal no. | | | W62551 | | | W62552 | |
|---|---|---|---|---|---|---|---|
| Test | Units | d-8 | d7 | d13 | d-8 | d7 | d13 |
| Na+ | mmol/l | 152 | 148 | 155 | 148 | 150 | 148 |
| K+ | mmol/l | 4.16 | 4.23 | 4.92 | 3.82 | 4.11 | 5.27 |
| Cl- | mmol/l | 110 | 105 | 111 | 109 | 106 | 108 |
| Ca++ | mmol/l | 2.64 | 2.61 | 2.61 | 2.48 | 2.44 | 1.80 |
| I.PHOS | mmol/l | 1.98 | 2.61 | 2.28 | 1.84 | 1.98 | 1.84 |
| Mg++ | mmol/l | 1.00 | 0.97 | 1.03 | 0.88 | 0.84 | 0.31 |
| GLUC | mmol/l | 3.65 | 8.39 | 3.86 | 2.79 | 3.86 | 3.60 |
| UREA | mmol/l | 11.0 | 8.3 | 8.2 | 11.3 | 6.9 | 6.3 |
| CREAT | µmol/l | 73 | 77 | 62 | 67 | 60 | 50 |
| TOT.BIL. | µmol/l | 4.00 | 2.00 | 3.00 | 5.00 | 1.00 | 2.00 |
| PROT | g/l | 85 | 80 | 80 | 83 | 83 | 77 |
| A/G | | 1.77 | 1.67 | 1.55 | 1.68 | 1.39 | 1.27 |
| CHOL | mmol/l | 3.20 | 2.80 | 3.00 | 3.70 | 3.40 | 3.50 |
| HDL-CHOL | mmol/l | 1.63 | 1.44 | 1.49 | 1.75 | 1.82 | 1.80 |
| LDL-CHOL | mmol/l | 1.55 | 1.25 | 1.90 | 1.57 | 1.28 | 1.66 |
| TRIG | mmol/l | 0.64 | 0.54 | 0.57 | 0.83 | 0.48 | 0.50 |
| ALP | IU/l | 1037 | 1088 | 1187 | 1332 | 1298 | 1182 |
| BAP-E | IU/l | 310 | 369 | 346 | 432 | 419 | 379 |
| ASAT | IU/l | 27 | 33 | 31 | 21 | 22 | 23 |
| ALAT | IU/l | 44 | 52 | 46 | 16 | 19 | 20 |
| CK | IU/l | 69 | 169 | 81 | 83 | 68 | 87 |
| LDH | IU/l | 420 | 520 | 481 | 474 | 471 | 516 |
| GGT | IU/l | 104 | 95 | 102 | 84 | 67 | 66 |
| ALB | % | 64 | 63 | 61 | 63 | 58 | 56 |
| A1-GLOB | % | 1.90 | 2.60 | 3.40 | 2.00 | 2.60 | 3.50 |
| A2-GLOB | % | 8.00 | 7.60 | 7.70 | 7.00 | 8.10 | 7.70 |
| B-GLOB | % | 17 | 18 | 18 | 15 | 18 | 18 |
| G-GLOB | % | 9.4 | 9.2 | 9.9 | 12.9 | 13.2 | 14.8 |
| ALB | g/l | 54 | 50 | 49 | 52 | 48 | 43 |
| A1-GLOB | g/l | 1.60 | 2.10 | 2.70 | 1.70 | 2.20 | 2.70 |
| A2-GLOB | g/l | 6.80 | 6.10 | 6.20 | 5.80 | 6.70 | 5.90 |
| B-GLOB | g/l | 14 | 14 | 15 | 13 | 15 | 14 |
| G-GLOB | g/l | 8.0 | 7.4 | 7.9 | 10.7 | 11.0 | 11.4 |

d-8, d7 and d13 indicate day -8, day 7 and day 13 relative to the starting day of dosing

## TABLE 29
## Clinical Chemistry – Females

### Salmon Calcitonin

| Animal no. | | W62553 | | | W62554 | | |
|---|---|---|---|---|---|---|---|
| Test | Units | d-8 | d7 | d13 | d-8 | d7 | d13 |
| Na+ | mmol/l | 145 | 147 | 147 | 145 | 143 | 147 |
| K+ | mmol/l | 3.51 | 3.73 | 4.62 | 3.89 | 4.07 | 4.95 |
| Cl- | mmol/l | 106 | 104 | 107 | 100 | 96 | 107 |
| Ca++ | mmol/l | 2.62 | 2.77 | 2.57 | 2.73 | 2.91 | 2.68 |
| I.PHOS | mmol/l | 1.62 | 1.48 | 1.81 | 1.97 | 1.75 | 1.83 |
| Mg++ | mmol/l | 0.87 | 0.63 | 0.76 | 0.91 | 0.77 | 0.80 |
| GLUC | mmol/l | 3.84 | 4.88 | 4.98 | 4.11 | 5.31 | 4.04 |
| UREA | mmol/l | 10.3 | 6.6 | 5.0 | 10.0 | 6.3 | 5.9 |
| CREAT | µmol/l | 81 | 71 | 61 | 88 | 77 | 65 |
| TOT.BIL. | µmol/l | 3.00 | 2.00 | 2.00 | 6.00 | 5.00 | 2.00 |
| PROT | g/l | 88 | 90 | 80 | 91 | 95 | 83 |
| A/G | | 1.46 | 1.45 | 1.30 | 1.48 | 1.42 | 1.26 |
| CHOL | mmol/l | 2.70 | 2.80 | 2.20 | 3.30 | 4.00 | 3.00 |
| HDL-CHOL | mmol/l | 1.04 | 1.11 | 0.96 | 1.46 | 1.99 | 1.66 |
| LDL-CHOL | mmol/l | 1.61 | 1.51 | 1.46 | 1.13 | 1.93 | 1.42 |
| TRIG | mmol/l | 0.79 | 0.25 | 0.39 | 0.88 | 0.30 | 0.38 |
| ALP | IU/l | 1197 | 965 | 842 | 1132 | 877 | 890 |
| BAP-E | IU/l | 416 | 326 | 304 | 344 | 325 | 294 |
| ASAT | IU/l | 24 | 21 | 25 | 20 | 18 | 20 |
| ALAT | IU/l | 21 | 24 | 19 | 19 | 14 | 19 |
| CK | IU/l | 99 | 72 | 107 | 76 | 64 | 77 |
| LDH | IU/l | 286 | 423 | 429 | 319 | 372 | 363 |
| GGT | IU/l | 88 | 63 | 54 | 82 | 72 | 62 |
| ALB | % | 59 | 59 | 57 | 60 | 59 | 56 |
| A1-GLOB | % | 2.70 | 2.70 | 3.10 | 2.20 | 2.20 | 3.10 |
| A2-GLOB | % | 6.50 | 6.10 | 6.80 | 8.00 | 7.70 | 7.80 |
| B-GLOB | % | 21 | 23 | 21 | 15 | 17 | 17 |
| G-GLOB | % | 10.8 | 8.6 | 12.4 | 14.9 | 14.6 | 16.3 |
| ALB | g/l | 52 | 54 | 45 | 54 | 56 | 46 |
| A1-GLOB | g/l | 2.40 | 2.40 | 2.50 | 2.00 | 2.10 | 2.60 |
| A2-GLOB | g/l | 5.70 | 5.50 | 5.40 | 7.30 | 7.30 | 6.50 |
| B-GLOB | g/l | 18 | 21 | 17 | 14 | 16 | 14 |
| G-GLOB | g/l | 9.5 | 7.7 | 9.9 | 13.6 | 13.9 | 13.5 |

d-8, d7 and d13 indicate day -8, day 7 and day 13 relative to the starting day of dosing

## TABLE 29
## Clinical Chemistry – Females

## PTS893

| Animal no. | | W62555 | | | W62556 | | |
|---|---|---|---|---|---|---|---|
| Test | Units | d-8 | d7 | d13 | d-8 | d7 | d13 |
| Na+ | mmol/l | 153 | 151 | 152 | 150 | 148 | 149 |
| K+ | mmol/l | 4.82 | 4.54 | 4.63 | 3.85 | 3.81 | 4.31 |
| Cl- | mmol/l | 107 | 109 | 111 | 108 | 107 | 114 |
| Ca++ | mmol/l | 2.77 | 2.61 | 2.20 | 2.64 | 2.62 | 2.35 |
| I.PHOS | mmol/l | 2.11 | 1.31 | 1.51 | 2.10 | 1.60 | 1.50 |
| Mg++ | mmol/l | 0.96 | 0.65 | 0.59 | 0.90 | 0.74 | 0.66 |
| GLUC | mmol/l | 3.57 | 4.18 | 3.59 | 3.22 | 4.45 | 3.52 |
| UREA | mmol/l | 8.2 | 8.7 | 6.3 | 8.4 | 6.6 | 6.8 |
| CREAT | µmol/l | 77 | 62 | 58 | 68 | 63 | 58 |
| TOT.BIL. | µmol/l | 5.00 | 1.00 | 2.00 | 5.00 | 2.00 | 2.00 |
| PROT | g/l | 89 | 87 | 78 | 84 | 83 | 76 |
| A/G | | 1.64 | 1.62 | 1.65 | 1.84 | 1.78 | 1.50 |
| CHOL | mmol/l | 2.90 | 2.70 | 2.80 | 2.70 | 2.40 | 2.70 |
| HDL-CHOL | mmol/l | 1.31 | 1.48 | 1.51 | 1.12 | 0.99 | 1.25 |
| LDL-CHOL | mmol/l | 1.69 | 1.12 | 1.71 | 1.62 | 1.28 | 1.58 |
| TRIG | mmol/l | 0.59 | 0.27 | 0.25 | 0.67 | 0.34 | 0.47 |
| ALP | IU/l | 1535 | 1223 | 1332 | 1638 | 1307 | 1313 |
| BAP-E | IU/l | 457 | 350 | 426 | 456 | 390 | 400 |
| ASAT | IU/l | 23 | 18 | 25 | 24 | 20 | 25 |
| ALAT | IU/l | 35 | 25 | 32 | 33 | 19 | 21 |
| CK | IU/l | 84 | 65 | 175 | 63 | 144 | 172 |
| LDH | IU/l | 468 | 465 | 557 | 309 | 313 | 358 |
| GGT | IU/l | 85 | 71 | 70 | 103 | 85 | 83 |
| ALB | % | 62 | 62 | 62 | 65 | 64 | 60 |
| A1-GLOB | % | 2.30 | 2.50 | 2.50 | 1.90 | 2.10 | 2.70 |
| A2-GLOB | % | 7.50 | 8.00 | 8.30 | 7.50 | 7.50 | 8.10 |
| B-GLOB | % | 18 | 19 | 18 | 17 | 17 | 20 |
| G-GLOB | % | 9.7 | 8.4 | 8.7 | 8.8 | 9.1 | 8.7 |
| ALB | g/l | 55 | 54 | 49 | 55 | 53 | 46 |
| A1-GLOB | g/l | 2.10 | 2.20 | 2.00 | 1.60 | 1.70 | 2.10 |
| A2-GLOB | g/l | 6.70 | 7.00 | 6.50 | 6.30 | 6.20 | 6.20 |
| B-GLOB | g/l | 16 | 17 | 14 | 14 | 14 | 16 |
| G-GLOB | g/l | 8.6 | 7.3 | 6.8 | 7.4 | 7.6 | 6.6 |

d-8, d7 and d13 indicate day -8, day 7 and day 13 relative to the starting day of dosing

[00159] No relevant changes were observed in the standard urinalysis tests performed.

TABLE 30
Urinary Analysis – Males

Control

| Animal no. | | | W62501 | | | W62502 | |
|---|---|---|---|---|---|---|---|
| Test | Units | -6 | -5 | 13 | -6 | -5 | 13 |
| VOLUME | ml | 15 | 10 | 77 | 22 | 130 | 30 |
| CREAT | µmol/l | 18000 | 17000 | 5460 | 7920 | 2480 | 5160 |
| NTx | nM BCE | - | 9954 | 3425 | - | 11979 | 3167 |
| CTx | µg/l | - | 21592 | 6810 | - | 27169 | 5323 |
| D-PYR | nmol/l | - | 2345 | 1110 | - | 2904 | 1461 |
| LDH | IU/L | 6.0 | | nd | 8.0 | | 8.0 |
| NAG | IU/l | 3.5 | | 1.5 | 3.2 | | 1.6 |
| Na+ | mmol/l | 163 | | 43 | 87 | | 77 |
| K+ | mmol/l | 258 | | 67 | 125 | | 75 |
| Cl- | mmol/l | 132 | | 43 | 52 | | 59 |
| Ca2+ | mmol/l | 5.15 | | 16.80 | 15.95 | | 15.50 |
| I.PHOS | mmol/l | 11.10 | | 1.05 | 11.30 | | 8.90 |
| Mg2+ | mmol/l | 2.75 | | 7.50 | 7.85 | | 6.25 |
| Na/Crea | mM/mM | 9.10 | | 7.90 | 11.00 | | 14.90 |
| K/Crea | mM/mM | 14.30 | | 12.20 | 15.80 | | 14.50 |
| Cl/Crea | mM/mM | 7.40 | | 7.90 | 6.50 | | 11.40 |
| Ca/Crea | mM/mM | 0.29 | | 3.08 | 2.01 | | 3.00 |
| Pho/Crea | mM/mM | 0.62 | | 0.19 | 1.43 | | 1.73 |
| Mg/Crea | mM/mM | 0.20 | | 1.40 | 1.00 | | 1.20 |
| LDH/crea | IU/mM | 0.33 | | nd | 1.01 | | 1.55 |
| NAG/crea | IU/mM | 0.19 | | 0.28 | 0.40 | | 0.31 |
| NTx/Crea | nME/mM | | 586 | 627 | | 4830 | 614 |
| CTx/Crea | µg/µm. | | 1270 | 1247 | | 10955 | 1032 |
| Pyr/Crea | nM/mM | | 138 | 203 | | 1171 | 283 |

d-6, d-5 and d13 indicate day -6, day -5 and day 13 relative to the starting day of dosing

## TABLE 30
## Urinary Analysis – Males

## Salmon Calcitonin

| Animal no. | | | W62503 | | | W62504 | |
|---|---|---|---|---|---|---|---|
| Test | Units | -6 | -5 | 13 | -6 | -5 | 13 |
| VOLUME | ml | 62 | 38 | 68 | 37 | 10 | 54 |
| CREAT | µmol/l | 4300 | 7840 | 4620 | 13600 | 17360 | 4400 |
| NTx | nM BCE | - | 6023 | 5186 | - | 16067 | 3790 |
| CTx | µg/l | - | 11618 | 10088 | - | 26370 | 6130 |
| D-PYR | nmol/l | - | 1733 | 1083 | - | 5113 | 1476 |
| LDH | IU/L | 9.0 | | 7.0 | 13.0 | | 17.0 |
| NAG | IU/l | 2.7 | | 1.4 | 4.2 | | 7.2 |
| Na+ | mmol/l | 22 | | 14 | 119 | | 15 |
| K+ | mmol/l | 65 | | 78 | 134 | | 76 |
| Cl- | mmol/l | 10 | | 55 | 64 | | 68 |
| Ca2+ | mmol/l | 0.90 | | 18.25 | 3.70 | | 23.40 |
| I.PHOS | mmol/l | 4.35 | | 2.50 | 5.33 | | 3.00 |
| Mg2+ | mmol/l | 1.40 | | 7.05 | 7.55 | | 9.80 |
| Na/Crea | mM/mM | 5.20 | | 3.10 | 8.70 | | 3.40 |
| K/Crea | mM/mM | 15.10 | | 16.90 | 9.90 | | 17.20 |
| Cl/Crea | mM/mM | 2.20 | | 11.80 | 4.70 | | 15.30 |
| Ca/Crea | mM/mM | 0.21 | | 3.95 | 0.27 | | 5.32 |
| Pho/Crea | mM/mM | 1.01 | | 0.54 | 0.39 | | 0.68 |
| Mg/Crea | mM/mM | 0.30 | | 1.50 | 0.60 | | 2.20 |
| LDH/crea | IU/mM | 2.09 | | 1.52 | 0.96 | | 3.86 |
| NAG/crea | IU/mM | 0.63 | | 0.30 | 0.31 | | 1.64 |
| NTx/Crea | nME/mM | | 768 | 1123 | | 926 | 861 |
| CTx/Crea | µg/µm. | | 1482 | 2184 | | 1519 | 1393 |
| Pyr/Crea | nM/mM | | 221 | 234 | | 295 | 336 |

d-6, d-5 and d13 indicate day -6, day -5 and day 13 relative to the starting day of dosing

## TABLE 30
## Urinary Analysis – Males

## PTS893

| Animal no. | | W62505 | | | W62506 | | |
|---|---|---|---|---|---|---|---|
| Test | Units | -6 | -5 | 13 | -6 | -5 | 13 |
| VOLUME | ml | 14 | 14 | 48 | 58 | 34 | 130 |
| CREAT | µmol/l | 16160 | 16160 | 7840 | 9940 | 16120 | 3840 |
| NTx | nM BCE | - | 5403 | 4871 | - | 8757 | 2102 |
| CTx | µg/l | - | 11865 | 9365 | - | 20108 | 3705 |
| D-PYR | nmol/l | - | 1660 | 1676 | - | 2278 | 782 |
| LDH | IU/L | 7.0 | | 14.0 | 9.0 | | 19.0 |
| NAG | IU/l | 23.4 | | 2.9 | 7.1 | | 2.6 |
| Na+ | mmol/l | 174 | | 111 | 59 | | 35 |
| K+ | mmol/l | 86 | | 107 | 125 | | 69 |
| Cl- | mmol/l | 22 | | 117 | 50 | | 48 |
| Ca2+ | mmol/l | 5.10 | | 7.55 | 3.50 | | 13.10 |
| I.PHOS | mmol/l | 74.40 | | 0.10 | 3.86 | | 0.17 |
| Mg2+ | mmol/l | 11.25 | | 8.70 | 2.95 | | 5.25 |
| Na/Crea | mM/mM | 10.80 | | 14.10 | 6.00 | | 9.10 |
| K/Crea | mM/mM | 5.30 | | 13.60 | 12.60 | | 17.90 |
| Cl/Crea | mM/mM | 1.40 | | 15.00 | 5.00 | | 12.60 |
| Ca/Crea | mM/mM | 0.32 | | 0.96 | 0.35 | | 3.41 |
| Pho/Crea | mM/mM | 4.60 | | 0.01 | 0.39 | | 0.04 |
| Mg/Crea | mM/mM | 0.70 | | 1.10 | 0.30 | | 1.40 |
| LDH/crea | IU/mM | 0.43 | | 1.79 | 0.91 | | 4.95 |
| NAG/crea | IU/mM | 1.45 | | 0.37 | 0.71 | | 0.68 |
| NTx/Crea | nME/mM | | 334 | 621 | | 543 | 547 |
| CTx/Crea | µg/µm. | | 734 | 1195 | | 1247 | 965 |
| Pyr/Crea | nM/mM | | 103 | 214 | | 141 | 204 |

d-6, d-5 and d13 indicate day -6, day -5 and day 13 relative to the starting day of dosing

## TABLE 31
## Urinary Analysis – Females

### Control

| Animal no. | | | W62551 | | | W62552 | |
|---|---|---|---|---|---|---|---|
| Test | Units | -8 | -7 | 13 | -8 | -7 | 13 |
| VOLUME | ml | 21 | 21 | 43 | 18 | 53 | 53 |
| CREAT | µmol/l | 16420 | 16420 | 9560 | 14300 | 6700 | 5380 |
| NTx | nM BCE | - | 9248 | 7824 | - | 5053 | 4695 |
| CTx | µg/l | - | 19280 | 17916 | - | 12014 | 10557 |
| D-PYR | nmol/l | - | 2500 | 2748 | - | 1397 | 2159 |
| LDH | IU/L | 10.0 | | 15.0 | 9.0 | | 25.0 |
| NAG | IU/l | 19.2 | | 4.2 | 10.3 | | 3.5 |
| Na+ | mmol/l | 110 | | 44 | 140 | | 64 |
| K+ | mmol/l | 82 | | 122 | 124 | | 87 |
| Cl- | mmol/l | 24 | | 73 | 72 | | 56 |
| Ca2+ | mmol/l | 2.90 | | 16.10 | 11.90 | | 19.50 |
| I.PHOS | mmol/l | 88.2 | | 7.7 | 20.3 | | 3.5 |
| Mg2+ | mmol/l | 2.35 | | 7.20 | 9.00 | | 5.45 |
| Na/Crea | mM/mM | 6.70 | | 4.60 | 9.80 | | 11.90 |
| K/Crea | mM/mM | 5.00 | | 12.80 | 8.70 | | 16.20 |
| Cl/Crea | mM/mM | 1.50 | | 7.60 | 5.10 | | 10.50 |
| Ca/Crea | mM/mM | 0.18 | | 1.68 | 0.83 | | 3.63 |
| Pho/Crea | mM/mM | 5.37 | | 0.81 | 1.42 | | 0.64 |
| Mg/Crea | mM/mM | 0.10 | | 0.80 | 0.60 | | 1.00 |
| LDH/crea | IU/mM | 0.61 | | 1.57 | 0.63 | | 4.65 |
| NAG/crea | IU/mM | 1.17 | | 0.44 | 0.72 | | 0.65 |
| NTx/Crea | nME/mM | | 563 | 818 | | 754 | 873 |
| CTx/Crea | µg/µm. | | 1174 | 1874 | | 1793 | 1962 |
| Pyr/Crea | nM/mM | | 152 | 288 | | 209 | 401 |

d-8, d-7 and d13 indicate day -8, day -7 and day 13 relative to the starting day of dosing

## TABLE 31
## Urinary Analysis – Females

## Salmon Calcitonin

| Animal no. | | | W62553 | | | W62554 | |
|---|---|---|---|---|---|---|---|
| Test | Units | -8 | -7 | 13 | -8 | -7 | 13 |
| VOLUME | ml | 11 | 58 | 67 | 32 | 14 | 49 |
| CREAT | µmol/l | 10780 | 6920 | 4800 | 11260 | 13380 | 4200 |
| NTx | nM BCE | - | 4624 | 3465 | - | 7393 | 2812 |
| CTx | µg/l | - | 6983 | 5392 | - | 13411 | 5631 |
| D-PYR | nmol/l | - | 2762 | 1644 | - | 2016 | 1110 |
| LDH | IU/L | 14.0 | | 6.0 | 6.0 | | 36.0 |
| NAG | IU/l | 10.2 | | 2.8 | 1.2 | | 2.7 |
| Na+ | mmol/l | 98 | | 40 | 156 | | 32 |
| K+ | mmol/l | 104 | | 53 | 172 | | 57 |
| Cl- | mmol/l | 31 | | 63 | 156 | | 65 |
| Ca2+ | mmol/l | 3.00 | | 17.55 | 3.50 | | 12.70 |
| I.PHOS | mmol/l | 25.4 | | 5.1 | 10.8 | | 5.8 |
| Mg2+ | mmol/l | 3.35 | | 5.40 | 3.80 | | 4.85 |
| Na/Crea | mM/mM | 9.10 | | 8.30 | 13.90 | | 7.60 |
| K/Crea | mM/mM | 9.60 | | 11.10 | 15.20 | | 13.50 |
| Cl/Crea | mM/mM | 2.90 | | 13.20 | 13.80 | | 15.40 |
| Ca/Crea | mM/mM | 0.28 | | 3.66 | 0.31 | | 3.02 |
| Pho/Crea | mM/mM | 2.35 | | 1.05 | 0.96 | | 1.38 |
| Mg/Crea | mM/mM | 0.30 | | 1.10 | 0.30 | | 1.20 |
| LDH/crea | IU/mM | 1.30 | | 1.25 | 0.53 | | 8.57 |
| NAG/crea | IU/mM | 0.95 | | 0.58 | 0.11 | | 0.64 |
| NTx/Crea | nME/mM | | 668 | 722 | | 553 | 670 |
| CTx/Crea | µg/µm. | | 1009 | 1123 | | 1002 | 1341 |
| Pyr/Crea | nM/mM | | 399 | 343 | | 151 | 264 |

d-8, d-7 and d13 indicate day -8, day -7 and day 13 relative to the starting day of dosing

## TABLE 31
## Urinary Analysis – Females

## PTS893

| Animal no. | | | W62555 | | | W62556 | |
|---|---|---|---|---|---|---|---|
| Test | Units | -8 | -7 | 13 | -8 | -7 | 13 |
| VOLUME | ml | 14 | 15 | 52 | 39 | 69 | 42 |
| CREAT | µmol/l | 19160 | 18240 | 5620 | 14060 | 7600 | 8060 |
| NTx | nM BCE | - | 10499 | 2514 | - | 4818 | 5679 |
| CTx | µg/l | - | 21919 | 3813 | - | 8877 | 11236 |
| D-PYR | nmol/l | - | 2963 | 1356 | - | 1377 | 2036 |
| LDH | IU/L | 11.0 | | 10.0 | 18.0 | | 9.0 |
| NAG | IU/l | 0.5 | | 1.2 | 5.9 | | 5.1 |
| Na+ | mmol/l | 145 | | 71 | 118 | | 146 |
| K+ | mmol/l | 302 | | 150 | 164 | | 70 |
| Cl- | mmol/l | 119 | | 101 | 53 | | 133 |
| Ca2+ | mmol/l | 11.50 | | 20.05 | 6.60 | | 12.35 |
| I.PHOS | mmol/l | 0.2 | | 0.1 | 7.6 | | 2.9 |
| Mg2+ | mmol/l | 7.35 | | 6.90 | 4.00 | | 5.90 |
| Na/Crea | mM/mM | 7.60 | | 12.60 | 8.40 | | 18.10 |
| K/Crea | mM/mM | 15.80 | | 26.80 | 11.70 | | 8.60 |
| Cl/Crea | mM/mM | 6.20 | | 18.00 | 3.70 | | 16.50 |
| Ca/Crea | mM/mM | 0.60 | | 3.57 | 0.47 | | 1.53 |
| Pho/Crea | mM/mM | 0.01 | | 0.02 | 0.54 | | 0.36 |
| Mg/Crea | mM/mM | 0.40 | | 1.20 | 0.30 | | 0.70 |
| LDH/crea | IU/mM | 0.57 | | 1.78 | 1.28 | | 1.12 |
| NAG/crea | IU/mM | 0.03 | | 0.21 | 0.42 | | 0.63 |
| NTx/Crea | nME/mM | | 576 | 447 | | 634 | 705 |
| CTx/Crea | µg/µm. | | 1202 | 679 | | 1168 | 1394 |
| Pyr/Crea | nM/mM | | 163 | 241 | | 181 | 253 |

d-8, d-7 and d13 indicate day -8, day -7 and day 13 relative to the starting day of dosing

[00160] The salmon calcitonin group presented with moderate decreases in serum somatomedin (S.MED, see TABLES 32 and 42).

TABLE 32
Hormones – Males

Control

| Animal no. | | | W62501 | | | W62502 | |
|---|---|---|---|---|---|---|---|
| Test | Units | d-6 | d7 | d13 | d-6 | d7 | d13 |
| ACTH | pg/ml | 91 | 63 | 87 | 117 | 136 | 150 |
| CORTISOL | nmol/l | 2183 | 1415 | 1328 | 1378 | 1020 | 1348 |
| ALDOST | pg/ml | 316 | 433 | 484 | 501 | 644 | 622 |
| INSULIN | mU/l | 26.0 | 33.0 | 37.0 | 12.0 | 30.0 | 9.0 |
| GLUCAG | pg/ml | 791 | 486 | 704 | 577 | 353 | 585 |
| C-PEPTI | ng/ml | n/a | 5.20 | 5.50 | n/a | 3.60 | 1.60 |
| GASTRIN | pg/ml | n/a | 105 | 93 | n/a | 147 | 148 |
| T3 | nmol/l | 1.34 | 2.61 | 2.94 | 2.19 | 2.73 | 2.50 |
| T4 | nmol/l | 56 | 61 | 44 | 57 | 68 | 48 |
| TSH | mUI/l | 0.17 | 0.18 | 0.42 | 0.00 | 0.05 | 0.04 |
| IPH | pg/ml | 103 | 75 | 108 | 174 | 173 | 155 |
| CT | pg/ml | 5.9 | 4.6 | 4.8 | 16.4 | 15.0 | 13.1 |
| VD25-H | nmol/l | 49 | 47 | 54 | 76 | 71 | 58 |
| VD1-25dh | pmol/l | n/a | - | - | n/a | - | - |
| OSTEO | ng/ml | n/a | 26 | 34 | n/a | 41 | 40 |
| CTx | nmol/l | 10 | 15 | 20 | 17 | 19 | 20 |
| ICTP | ng/ml | 18 | 13 | 19 | 26 | 16 | 15 |
| PICP | ng/ml | n/a | 311 | 395 | n/a | 610 | 495 |
| G.H. | ng/ml | 13.8 | 7.0 | 16.2 | 15.2 | 3.6 | 17.2 |
| S.STA | pg/ml | n/a | - | - | n/a | - | - |
| S.MED | ng/ml | n/a | 888 | 1185 | n/a | 793 | 689 |
| PROLACT | ng/ml | 0.0 | 3.3 | 3.6 | 21.6 | 22.5 | 22.5 |
| TESTO | nmol/l | 10.5 | 8.4 | n. s. | 7.9 | 4.7 | n. s. |
| ESTR | nmol/l | n/a | n/a | n/a | n/a | n/a | n/a |
| PROG | pmol/l | n/a | n/a | n/a | n/a | n/a | n/a |

d-6, d7 and d13 indicate day -6, day 7 and day 13 relative to the starting day of dosing

[00160] The salmon calcitonin group presented with moderate decreases in serum somatomedin (S.MED, see TABLES 32 and 42).

TABLE 32
Hormones – Males

Control

| Animal no. | | | W62501 | | | W62502 | |
|---|---|---|---|---|---|---|---|
| Test | Units | d-6 | d7 | d13 | d-6 | d7 | d13 |
| ACTH | pg/ml | 91 | 63 | 87 | 117 | 136 | 150 |
| CORTISOL | nmol/l | 2183 | 1415 | 1328 | 1378 | 1020 | 1348 |
| ALDOST | pg/ml | 316 | 433 | 484 | 501 | 644 | 622 |
| INSULIN | mU/l | 26.0 | 33.0 | 37.0 | 12.0 | 30.0 | 9.0 |
| GLUCAG | pg/ml | 791 | 486 | 704 | 577 | 353 | 585 |
| C-PEPTI | ng/ml | n/a | 5.20 | 5.50 | n/a | 3.60 | 1.60 |
| GASTRIN | pg/ml | n/a | 105 | 93 | n/a | 147 | 148 |
| T3 | nmol/l | 1.34 | 2.61 | 2.94 | 2.19 | 2.73 | 2.50 |
| T4 | nmol/l | 56 | 61 | 44 | 57 | 68 | 48 |
| TSH | mUI/l | 0.17 | 0.18 | 0.42 | 0.00 | 0.05 | 0.04 |
| IPH | pg/ml | 103 | 75 | 108 | 174 | 173 | 155 |
| CT | pg/ml | 5.9 | 4.6 | 4.8 | 16.4 | 15.0 | 13.1 |
| VD25-H | nmol/l | 49 | 47 | 54 | 76 | 71 | 58 |
| VD1-25dh | pmol/l | n/a | - | - | n/a | - | - |
| OSTEO | ng/ml | n/a | 26 | 34 | n/a | 41 | 40 |
| CTx | nmol/l | 10 | 15 | 20 | 17 | 19 | 20 |
| ICTP | ng/ml | 18 | 13 | 19 | 26 | 16 | 15 |
| PICP | ng/ml | n/a | 311 | 395 | n/a | 610 | 495 |
| G.H. | ng/ml | 13.8 | 7.0 | 16.2 | 15.2 | 3.6 | 17.2 |
| S.STA | pg/ml | n/a | - | - | n/a | - | - |
| S.MED | ng/ml | n/a | 888 | 1185 | n/a | 793 | 689 |
| PROLACT | ng/ml | 0.0 | 3.3 | 3.6 | 21.6 | 22.5 | 22.5 |
| TESTO | nmol/l | 10.5 | 8.4 | n. s. | 7.9 | 4.7 | n. s. |
| ESTR | nmol/l | n/a | n/a | n/a | n/a | n/a | n/a |
| PROG | pmol/l | n/a | n/a | n/a | n/a | n/a | n/a |

d-6, d7 and d13 indicate day -6, day 7 and day 13 relative to the starting day of dosing

## TABLE 32
## Hormones – Males

## Salmon Calcitonin

| Animal no. | | | W62503 | | | W62504 | |
|---|---|---|---|---|---|---|---|
| Test | Units | d-6 | d7 | d13 | d-6 | d7 | d13 |
| ACTH | pg/ml | 98 | 87 | 87 | 115 | 78 | 73 |
| CORTISOL | nmol/l | 2316 | 979 | 1611 | 1578 | 1523 | 1709 |
| ALDOST | pg/ml | 983 | 1058 | 819 | 465 | 987 | 977 |
| INSULIN | mU/l | 13.0 | 14.0 | 17.0 | 4.0 | 10.0 | 22.0 |
| GLUCAG | pg/ml | 905 | 247 | 428 | 869 | 218 | 503 |
| C-PEPTI | ng/ml | n/a | 1.70 | 1.80 | n/a | 1.20 | 2.30 |
| GASTRIN | pg/ml | n/a | 83 | 88 | n/a | 128 | 136 |
| T3 | nmol/l | 1.06 | 2.35 | 2.51 | 1.48 | 1.65 | 1.90 |
| T4 | nmol/l | 53 | 64 | 47 | 62 | 79 | 65 |
| TSH | mUI/l | 0.99 | 1.12 | 1.03 | 0.14 | 0.41 | 0.40 |
| IPH | pg/ml | 213 | 75 | 78 | 99 | 62 | 71 |
| CT | pg/ml | 6.7 | 4.0 | 2.4 | 5.1 | 2.5 | 4.9 |
| VD25-H | nmol/l | 63 | 50 | 49 | 62 | 44 | 45 |
| VD1-25dh | pmol/l | n/a | - | - | n/a | - | - |
| OSTEO | ng/ml | n/a | 33 | 41 | n/a | 27 | 30 |
| CTx | nmol/l | 12 | 26 | 38 | 18 | 22 | 24 |
| ICTP | ng/ml | 21 | 15 | 15 | 22 | 21 | 20 |
| PICP | ng/ml | n/a | 284 | 363 | n/a | 361 | 439 |
| G.H. | ng/ml | 11.5 | 1.7 | 16.2 | 14.6 | 13.6 | 15.7 |
| S.STA | pg/ml | n/a | - | - | n/a | - | - |
| S.MED | ng/ml | n/a | 268 | 332 | n/a | 307 | 384 |
| PROLACT | ng/ml | 8.1 | 8.6 | 4.6 | 0.0 | 0.0 | 6.6 |
| TESTO | nmol/l | 8.5 | 3.6 | n. s. | 9.5 | 7.3 | n. s. |
| ESTR | nmol/l | n/a | n/a | n/a | n/a | n/a | n/a |
| PROG | pmol/l | n/a | n/a | n/a | n/a | n/a | n/a |

d-6, d7 and d13 indicate day -6, day 7 and day 13 relative to the starting day of dosing

## TABLE 32
## Hormones – Males

## PTS893

| Animal no. | | | W62505 | | | W62506 | |
|---|---|---|---|---|---|---|---|
| Test | Units | d-6 | d7 | d13 | d-6 | d7 | d13 |
| ACTH | pg/ml | 96 | 101 | 83 | 115 | 88 | 91 |
| CORTISOL | nmol/l | 1662 | 1156 | 1299 | 1506 | 1432 | 1212 |
| ALDOST | pg/ml | 265 | 380 | 592 | 141 | 471 | 651 |
| INSULIN | mU/l | 16.0 | 22.0 | 14.0 | 12.0 | 38.0 | 10.0 |
| GLUCAG | pg/ml | 858 | 656 | 786 | 694 | 497 | 739 |
| C-PEPTI | ng/ml | n/a | 2.90 | 2.10 | n/a | 4.40 | 2.40 |
| GASTRIN | pg/ml | n/a | 84 | 78 | n/a | 98 | 94 |
| T3 | nmol/l | 2.48 | 3.47 | 3.55 | 1.38 | 2.76 | 2.43 |
| T4 | nmol/l | 84 | 90 | 68 | 59 | 80 | 56 |
| TSH | mUI/l | 0.22 | 0.40 | 0.15 | 0.00 | 0.07 | 0.03 |
| IPH | pg/ml | 123 | 96 | 78 | 71 | 62 | 55 |
| CT | pg/ml | 6.1 | 4.0 | 4.6 | 10.4 | 7.8 | 7.6 |
| VD25-H | nmol/l | 77 | 62 | 50 | 88 | 62 | 50 |
| VD1-25dh | pmol/l | n/a | - | - | n/a | - | - |
| OSTEO | ng/ml | n/a | 43 | 55 | n/a | 32 | 42 |
| CTx | nmol/l | 19 | 20 | 31 | 12 | 12 | 16 |
| ICTP | ng/ml | 28 | 23 | 22 | 18 | 16 | 18 |
| PICP | ng/ml | n/a | 420 | 500 | n/a | 774 | 706 |
| G.H. | ng/ml | 13.4 | 15.8 | 12.1 | 8.5 | 11.6 | 14.0 |
| S.STA | pg/ml | n/a | - | - | n/a | - | - |
| S.MED | ng/ml | n/a | 749 | 914 | n/a | 828 | 867 |
| PROLACT | ng/ml | 7.1 | 15.7 | 7.5 | 7.5 | 5.5 | 2.2 |
| TESTO | nmol/l | 11.8 | 10.5 | n. s. | 5.3 | 3.7 | n. s. |
| ESTR | nmol/l | n/a | n/a | n/a | n/a | n/a | n/a |
| PROG | pmol/l | n/a | n/a | n/a | n/a | n/a | n/a |

d-6, d7 and d13 indicate day -6, day 7 and day 13 relative to the starting day of dosing

## TABLE 33
## Hormones – Females

### Control

| Animal no. | | | W62551 | | | W62552 | |
|---|---|---|---|---|---|---|---|
| Test | Units | d-8 | d7 | d13 | d-8 | d7 | d13 |
| ACTH | pg/ml | 146 | 276 | 121 | 58 | 60 | 101 |
| CORTISOL | nmol/l | 1983 | 1546 | 827 | 1894 | 837 | 818 |
| ALDOST | pg/ml | 244 | 953 | 312 | 149 | 90 | 199 |
| INSULIN | mU/l | 8.0 | 12.0 | 7.0 | 2.0 | 29.0 | 21.0 |
| GLUCAG | pg/ml | 729 | 779 | 583 | 818 | 507 | 514 |
| C-PEPTI | ng/ml | n/a | 2.40 | 1.40 | n/a | 3.30 | 2.30 |
| GASTRIN | pg/ml | n/a | 84 | 102 | n/a | 90 | 92 |
| T3 | nmol/l | 2.22 | 2.95 | 3.40 | 2.04 | 3.09 | 3.23 |
| T4 | nmol/l | 78 | 67 | 59 | 51 | 50 | 49 |
| TSH | mUl/l | 0.14 | 0.27 | 0.49 | 0.15 | 0.54 | 0.50 |
| IPH | pg/ml | 155 | 149 | 129 | 145 | 129 | 112 |
| CT | pg/ml | 4.70 | 3.90 | 4.10 | 11.50 | 11.60 | 11.20 |
| VD25-H | nmol/l | 64 | 59 | 51 | 80 | 78 | 70 |
| VD1-25dh | pmol/l | n/a | - | - | n/a | - | - |
| OSTEO | ng/ml | n/a | 37 | 39 | n/a | 34 | 39 |
| CTx | nmol/l | 11 | 26 | 28 | 12 | 16 | 20 |
| ICTP | ng/ml | 21 | 23 | 22 | 19 | 16 | 15 |
| PICP | ng/ml | n/a | 864 | 503 | n/a | 339 | 298 |
| G.H. | ng/ml | 8.5 | 13.4 | 1.7 | 7.0 | 12.0 | 4.5 |
| S.STA | pg/ml | n/a | - | - | n/a | - | - |
| S.MED | ng/ml | n/a | 696 | 839 | n/a | 1173 | 1527 |
| PROLACT | ng/ml | 4.30 | 8.30 | 5.90 | 2.90 | 0.00 | 0.00 |
| TESTO | nmol/l | | | | | | |
| ESTR | nmol/l | 58 | 64 | 61 | 48 | 45 | 60 |
| PROG | pmol/l | 3.40 | 3.50 | 1.70 | 2.70 | 1.10 | 1.40 |

d-8, d7 and d13 indicate day -8, day 7 and day 13 relative to the starting day of dosing

## TABLE 33
## Hormones – Females

## Salmon Calcitonin

| Animal no. | | | W62553 | | | W62554 | |
|---|---|---|---|---|---|---|---|
| Test | Units | d-8 | d7 | d13 | d-8 | d7 | d13 |
| ACTH | pg/ml | 72 | 129 | 97 | 157 | 233 | 141 |
| CORTISOL | nmol/l | 1536 | 1220 | 1202 | 1222 | 1705 | 1128 |
| ALDOST | pg/ml | 185 | 948 | 523 | 155 | 1073 | 457 |
| INSULIN | mU/l | 12.0 | 8.0 | 9.0 | 20.0 | 18.0 | 24.0 |
| GLUCAG | pg/ml | 585 | 295 | 258 | 619 | 594 | 303 |
| C-PEPTI | ng/ml | n/a | 1.60 | 1.00 | n/a | 1.50 | 2.20 |
| GASTRIN | pg/ml | n/a | 83 | 84 | n/a | 91 | 84 |
| T3 | nmol/l | 1.17 | 1.68 | 1.51 | 1.43 | 1.51 | 2.00 |
| T4 | nmol/l | 58 | 76 | 60 | 61 | 87 | 60 |
| TSH | mUI/l | 0.81 | 1.31 | 1.16 | 0.08 | 0.34 | 0.41 |
| IPH | pg/ml | 59 | 47 | 58 | 145 | 82 | 53 |
| CT | pg/ml | 3.10 | 6.40 | 4.90 | 7.00 | 3.60 | 2.30 |
| VD25-H | nmol/l | 61 | 43 | 40 | 72 | 56 | 60 |
| VD1-25dh | pmol/l | n/a | - | - | n/a | - | - |
| OSTEO | ng/ml | n/a | 21 | 25 | n/a | 35 | 35 |
| CTx | nmol/l | 12 | 21 | 25 | 17 | 34 | 28 |
| ICTP | ng/ml | 28 | 28 | 24 | 29 | 30 | 24 |
| PICP | ng/ml | n/a | 115 | 142 | n/a | 240 | 287 |
| G.H. | ng/ml | 6.3 | 15.2 | 8.6 | 5.1 | 17.9 | 13.1 |
| S.STA | pg/ml | n/a | - | - | n/a | - | - |
| S.MED | ng/ml | n/a | 374 | 297 | n/a | 204 | 488 |
| PROLACT | ng/ml | 0.00 | 2.30 | 4.30 | 19.30 | 20.20 | 24.40 |
| TESTO | nmol/l | | | | | | |
| ESTR | nmol/l | 47 | 63 | 59 | 141 | 82 | 170 |
| PROG | pmol/l | 1.80 | 1.90 | 1.50 | 2.60 | 4.00 | 1.60 |

d-8, d7 and d13 indicate day -8, day 7 and day 13 relative to the starting day of dosing

TABLE 33
Hormones – Females

PTS893

| Animal no. | | | W62555 | | | W62556 | |
|---|---|---|---|---|---|---|---|
| Test | Units | d-8 | d7 | d13 | d-8 | d7 | d13 |
| ACTH | pg/ml | 109 | 104 | 110 | 95 | 132 | 126 |
| CORTISOL | nmol/l | 1482 | 1331 | 917 | 1532 | 1253 | 1375 |
| ALDOST | pg/ml | 314 | 217 | 330 | 210 | 228 | 226 |
| INSULIN | mU/l | 1.0 | 22.0 | 19.0 | 15.0 | 30.0 | 22.0 |
| GLUCAG | pg/ml | 711 | 591 | 657 | 696 | 437 | 380 |
| C-PEPTI | ng/ml | n/a | 3.00 | 2.40 | n/a | 3.80 | 3.50 |
| GASTRIN | pg/ml | n/a | 83 | 82 | n/a | 96 | 91 |
| T3 | nmol/l | 2.08 | 2.74 | 2.63 | 1.98 | 2.69 | 2.05 |
| T4 | nmol/l | 72 | 56 | 55 | 59 | 61 | 45 |
| TSH | mUI/l | 0.34 | 0.14 | 0.25 | 0.88 | 0.89 | 0.69 |
| IPH | pg/ml | 95 | 45 | 64 | 111 | 67 | 58 |
| CT | pg/ml | 2.50 | 1.90 | 2.70 | 1.80 | 2.90 | 2.80 |
| VD25-H | nmol/l | 72 | 53 | 47 | 55 | 44 | 43 |
| VD1-25dh | pmol/l | n/a | - | - | n/a | - | - |
| OSTEO | ng/ml | n/a | 38 | 43 | n/a | 32 | 36 |
| CTx | nmol/l | 13 | 11 | 15 | 17 | 14 | 14 |
| ICTP | ng/ml | 22 | 16 | 16 | 20 | 15 | 15 |
| PICP | ng/ml | n/a | 612 | 436 | n/a | 478 | 393 |
| G.H. | ng/ml | 3.5 | 1.5 | 0.0 | 1.1 | 8.2 | 11.8 |
| S.STA | pg/ml | n/a | - | - | n/a | - | - |
| S.MED | ng/ml | n/a | 533 | 502 | n/a | 432 | 589 |
| PROLACT | ng/ml | 0.00 | 0.20 | 3.20 | 9.90 | 5.70 | 3.60 |
| TESTO | nmol/l | | | | | | |
| ESTR | nmol/l | 67 | 68 | 60 | 59 | 66 | 57 |
| PROG | pmol/l | 2.80 | 1.70 | 1.50 | 2.40 | 2.20 | 2.40 |

d-8, d7 and d13 indicate day -8, day 7 and day 13 relative to the starting day of dosing

[00161] *Tissue sampling*. Animals were killed by deep anaesthesia induced by intravenous injection of Pentothal®, followed by exsanguinations. All relevant tissues were sampled for histopathology and gene expression profiling. The following tissue samples were processed for analysis: liver, kidney, pituitary, muscle, bone, duodenum, spleen and trachea. Samples for histopathology were fixed in phosphate-buffered 10% formalin. Bone demineralization was performed with 10% formic acid. Tissue samples were embedded in Paraplast® and sectioned at 4 microns, for staining with haematoxylin and eosin. Samples for gene expression profiling were quickly frozen in liquid nitrogen immediately after excision, stored on dry ice and subsequently in a deep-freezer at approximately -80°C until further use. All selected tissues for gene expression profiling were examined histopathologically.

[00162] *Histopathology*. Histopathological examination of the tissues selected for gene profiling analysis exhibited a normal spectrum of incidental lesions which were in terms of severity and distribution of lesions not different to the controls in all groups of treatment.

[00163] A slightly higher incidence of inflammatory and regenerative changes in the kidneys of females administered salmon calcitonin was observed. These changes were not considered to be relevant, since no records of kidney toxicity exist after 40 years of calcitonin therapeutic use.

[00164] Bone sections were stained for osteonectin, osteopontin and osteocalcin and were evaluated histopathologically. Histomorphometry of the bone tissue was performed regarding parameters for bone resorption and synthesis (osteoid formation).

[00165] The osteonectin, osteopontin, and osteocalcin staining of the tibia showed no difference between the groups one (control) and two (salmon calcitonin). Osteonectin exhibited a major enlargement and deterioration of the epiphysial growth plate of animal no 2553 due to a severe non-treatment related pathological status (severe, subacute epiphysiolysis).

[00166] Histomorphometry of bone tissue was performed to determine parameters related to bone resorption and bone synthesis (osteoid formation).

[00167] The results (see, TABLES 34 and 35) showed that salmon calcitonin increased trabecular volume and thickness in about a 17% in tibia, but not in vertebra. PTS893 reduced the cortical thickness (18%) and increased the cortical porosity (54%) in tibia (T), but not in vertebra (V). In contrast, PTS893 induced an increase in osteoid volume (37%T, 213%V) and surface (49%T, 37%V), as well as an increase in the osteoblast surface (40%T, 24%V), in both tibia and vertebra, respectively.

## TABLE 34
## Histomorphometry Tibia (Average Males and Females)

| | BT/TV % | Tb Th µm | Tb N $mm^{-1}$ | Tb Sp µm | Ct Por % | Ct Th µm | OS/BS % | OV/BV % | ES/BS % | Obs/BS % |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 20.70 | 106.32 | 1.95 | 407.20 | 2.53 | 1583.13 | 40.00 | 8.76 | 5.73 | 17.53 |
| | 17.72 | 97.99 | 1.81 | 454.90 | 2.59 | 976.66 | 33.37 | 8.51 | 4.70 | 12.77 |
| | 28.74 | 109.18 | 2.63 | 270.69 | 1.21 | 1036.24 | 29.45 | 5.79 | 10.19 | 11.70 |
| | 20.15 | 103.59 | 1.94 | 410.62 | 1.19 | 1031.89 | 29.19 | 5.29 | 5.71 | 15.80 |
| mean | 21.83 | 104.27 | 2.08 | 385.85 | 1.88 | 1156.98 | 33.00 | 7.09 | 6.58 | 14.45 |
| SD | 4.79 | 4.77 | 0.37 | 79.79 | 0.78 | 285.39 | 5.04 | 1.80 | 2.45 | 2.69 |
| sCT | 32.28 | 140.64 | 2.30 | 295.01 | 2.10 | 895.98 | 42.71 | 11.72 | 5.02 | 18.32 |
| | 25.00 | 122.19 | 2.05 | 366.51 | 1.98 | 1022.55 | 31.58 | 5.86 | 2.31 | 6.37 |
| | 29.96 | 129.05 | 2.32 | 301.75 | 1.61 | 939.32 | 35.21 | 5.03 | 6.89 | 18.58 |
| | 16.08 | 115.65 | 1.39 | 603.45 | 2.40 | 1178.70 | 30.37 | 4.01 | 5.61 | 19.36 |
| mean | 25.83 | 126.88 | 2.01 | 391.68 | 2.02 | 1009.14 | 34.97 | 6.65 | 4.95 | 15.66 |
| SD | 7.17 | 10.68 | 0.43 | 144.81 | 0.33 | 124.65 | 5.56 | 3.46 | 1.93 | 6.21 |
| PTS893 | 19.69 | 129.22 | 1.52 | 526.99 | 2.76 | 1022.62 | 54.84 | 11.24 | 4.62 | 16.16 |
| | 16.65 | 93.20 | 1.79 | 466.69 | 2.94 | 893.43 | 43.57 | 9.61 | 4.76 | 21.25 |
| | 25.74 | 120.52 | 2.13 | 347.63 | 2.94 | 950.33 | 43.63 | 8.14 | 4.21 | 18.46 |
| | 24.78 | 126.07 | 1.97 | 382.61 | 2.95 | 939.53 | 54.97 | 9.95 | 2.85 | 25.25 |
| mean | 21.72 | 117.25 | 1.85 | 430.98 | 2.90 | 951.48 | 49.25 | 9.74 | 4.11 | 20.28 |
| SD | 4.30 | 16.43 | 0.26 | 81.20 | 0.09 | 53.46 | 6.53 | 1.28 | 0.87 | 3.91 |

sCT: salmon Calcitonin; SD: Standard deviation
BV/TV trabecular bone volume; Tb. Th. Trabecular thickness; Tb. N. Trabecular number; Tb. Sp. Trabecular Separation; Ct. Por. Cortical porosity; Ct, Th. Cortical thickness; OS/BS osteoid surface; OV/BV osteoid volume; ES/BS eroded surface; Obs/BS osteoblast surface.

## TABLE 35
## Histomorphometry Vertebra (Average Males and Females)

| | BT/TV % | Tb Th µm | Tb N $mm^{-1}$ | Tb Sp µm | Ct Por % | Ct Th µm | OS/BS % | OV/BV % | ES/BS % | Obs/BS % |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 21.67 | 179.80 | 1.21 | 649.79 | 0.88 | 887.91 | 23.61 | 1.22 | 8.94 | 16.14 |
| | 15.85 | 144.89 | 1.09 | 769.35 | 0.26 | 639.93 | 20.77 | 2.02 | 8.81 | 5.96 |
| | 19.54 | 122.91 | 1.59 | 506.23 | 0.87 | 416.48 | 17.91 | 1.58 | 5.85 | 4.07 |
| | 21.95 | 131.30 | 1.67 | 466.91 | 0.85 | 604.45 | 11.58 | 0.97 | 1.82 | 4.79 |
| mean | 19.75 | 144.72 | 1.39 | 598.07 | 0.71 | 637.20 | 18.47 | 1.45 | 6.36 | 7.74 |
| SD | 2.82 | 25.07 | 0.28 | 138.62 | 0.30 | 193.78 | 5.15 | 0.45 | 3.34 | 5.65 |
| sCT | 17.32 | 113.29 | 1.53 | 540.84 | 1.70 | 705.10 | 3.95 | 0.46 | 11.60 | 3.21 |
| | 19.33 | 144.31 | 1.34 | 602.15 | 1.18 | 810.09 | 5.82 | 0.86 | 2.55 | 3.97 |
| | 20.11 | 118.49 | 1.70 | 470.71 | 1.18 | 576.42 | 11.48 | 1.43 | 4.93 | 6.81 |
| | 19.46 | 123.71 | 1.57 | 511.96 | 0.12 | 907.16 | 4.91 | 0.32 | 3.47 | 1.23 |
| mean | 19.06 | 124.95 | 1.53 | 531.42 | 1.05 | 749.69 | 6.54 | 0.77 | 5.64 | 3.80 |
| SD | 1.21 | 13.59 | 0.15 | 55.24 | 0.66 | 141.96 | 3.38 | 0.50 | 4.09 | 2.31 |
| PTS893 | 15.15 | 105.46 | 1.44 | 590.67 | 1.49 | 707.43 | 18.84 | 3.24 | 9.31 | 10.36 |
| | 20.23 | 118.79 | 1.70 | 468.39 | 1.45 | 629.35 | 41.28 | 8.42 | 2.30 | 9.07 |
| | 23.56 | 134.66 | 1.75 | 436.79 | 0.41 | 740.87 | 23.65 | 3.49 | 2.55 | 10.47 |
| | 24.86 | 134.82 | 1.84 | 407.56 | 0.92 | 624.35 | 17.66 | 2.66 | 3.96 | 8.33 |
| mean | 20.95 | 123.43 | 1.68 | 475.85 | 1.07 | 675.50 | 25.36 | 4.45 | 4.53 | 9.56 |
| SD | 4.33 | 14.15 | 0.17 | 80.47 | 0.51 | 57.85 | 10.93 | 2.67 | 3.27 | 1.04 |

sCT: salmon Calcitonin; SD: Standard deviation
BV/TV trabecular bone volume; Tb. Th. Trabecular thickness; Tb. N. Trabecular number; Tb. Sp. Trabecular Separation; Ct. Por. Cortical porosity; Ct, Th. Cortical thickness; OS/BS osteoid surface; OV/BV osteoid volume; ES/BS eroded surface; Obs/BS osteoblast surface.

[00168] Histomorphometry showed inconsistent results between tibial and vertebral bone, except for an increase in osteoid synthesis induced by PTS893. This effect is well documented for parathyroid hormone, when administered in a discontinuous way.

[00169] *RNA extraction and purification.* A set of tissues was selected for gene expression profiling. These set included samples from kidney, bone, muscle, duodenum, pituitary and liver. Briefly, total RNA was obtained by acid guanidinium thiocyanate-phenol-chloroform extraction (Trizol®, Invitrogen Life Technologies, Carlsbad, Calif. USA) from each frozen tissue section and the total RNA was then purified on an affinity resin (RNeasy®, Qiagen) according to the manufacturer's instructions. Total RNA was quantified by the absorbance at $\lambda = 260$ nm (A260nm), and the purity was estimated by the ratio $A_{260nm}/A_{280nm}$. Integrity of the RNA molecules was confirmed by non-denaturing agarose gel electrophoresis. RNA was stored at approximately -80°C until analysis. One part of each individual RNA sample was kept for the analysis of critical genes by means of Real-time PCR.

[00170] *Hybridization assay.* Transcript profiling by means of GeneChip® expression probe arrays was done as recommended by the manufacturer of the GeneChip® system (*GeneChip Expression Analysis Technical Manual*, Affymetrix Inc., Santa Clara, Calif. USA). HG-U95Av2 GeneChip® expression probe arrays (Affymetrix, Santa Clara Calif. USA) were used. Double stranded cDNA was synthesized with a starting amount of approximately 5 µg full-length total RNA using the Superscript Choice System (Invitrogen Life Technologies) in the presence of a T7-(dT) 24 DNA oligonucleotide primer. Following synthesis, the cDNA was purified by phenol/chloroform/isoamylalcohol extraction and ethanol precipitation. The purified cDNA was then transcribed in vitro using the BioArray® High Yield RNA Transcript Labeling Kit (ENZO) in the presence of biotinylated ribonucleotides form biotin labeled cRNA. The labeled cRNA was then purified on an affinity resin (Rneasy®, Qiagen), quantified and fragmented. An amount of approximately 10 µg labeled cRNA was hybridized for approximately 16 hours at 45°C to an expression probe array. The array was then washed and stained twice with streptavidin-phycoerythrin (Molecular Probes) using the GeneChip Fluidics Workstation 400 (Affymetrix). The array was then scanned twice using a confocal laser scanner (GeneArray® Scanner, Agilent) resulting in one scanned image.

[00171] This resulting ".data-file" was processed using the Micro Array Analysis Suite version 4 (MAS4) program (Affymetrix) into a ".cel-file". The ".cel file" was captured and loaded into the Affymetrix GeneChip Laboratory Information Management System (LIMS).

The LIMS database is connected to a UNIX Sun Solaris server through a network filing system that allows for the average intensities for all probes cells (CEL file) to be downloaded into an Oracle database. Raw data was converted to expression levels using a "target intensity" of 150. The numerical values displayed are weighted averages of the signal intensities of the probe-pairs comprised in a probe-set for a given transcript sequence (AvgDiff value). The data were checked for quality and loaded into the GeneSpring® software versions 4.2.4 and 5 (Silicon Genetics, Calif. USA) for analysis.

[00172] *Data analysis*. Data analysis was performed with the Silicon Genetics software package GeneSpring version 4.2.1 and 5. Average difference values below 20 were set to 20. Various filtering and clustering tools in these programs were used to explore the data sets and identify transcript level changes that inform on altered cellular and tissue functions and that can be used to establish working hypotheses on the modes of action of the compound.

[00173] The threshold range for considering as up or down regulation was determined within the context of the biological interpretation of the study.

[00174] The information content of these data sets is a conjunction of numerical changes and biological information. The decision to consider a specific gene relevant was based on a conjunction of numerical changes identified by comparative and statistical algorithms and the relationship to other modulated genes that point to a common biological theme. The weight of that relationship was assessed by the analyst through a review of the relevant scientific literature.

[00175] Increase and decrease reported here refer to transcript abundance, unless specifically stated.

[00176] *Gene expression profiling.* Multiorgan comparative gene profiling analysis was performed in the group administered salmon calcitonin at 50 µg/animal/day. The organs chosen for analysis were liver, kidney, pituitary, skeletal muscle, bone, duodenum, spleen and trachea.

TABLE 36

Multi-Organ Gene Expression Profiling of Salmon Calcitonin

| GeneChip® expression probe set identifier | Coding Gene | bone | kidney | liver | muscle | pituitary | trachea |
|---|---|---|---|---|---|---|---|
| 36611_at | acid phosphatase 1 isoform a | | | -1.33 | | -1.33 | |
| 32714_s_at | activin A receptor type II-like 1 | -1.62 | | | -1.83 | | |
| 39314_at | activin A type IIB receptor precursor | | | -1.12 | 1.41 | -4.15 | |
| 35915_at | activin beta-C chain. | -1.21 | | | -2.41 | -1.67 | |
| 36621_at | alpha-2-HS-glycoprotein | 1.33 | 1.53 | | | | 1.12 |
| 34588_i_at | amelogenin | | | -1.61 | | | |
| 37747_at | annexin V | | -1.30 | 1.87 | | -2.58 | |
| 40376_at | arylsulfatase E precursor | | -1.59 | | | | |
| 39326_at | ATPase H(+)- vacuolar | -1.57 | -2.80 | | | -1.62 | |
| 38814_at | ATPase H(+)- vacuolar subunit | 1.22 | | | | | |
| 33741_at | ATPase, H+ transport, lysosomal | 1.23 | | | | -1.50 | |
| 33033_at | ATPase, H+ transporting, lysosomal | -1.29 | | -3.19 | | -1.43 | 1.23 |
| 38814_at | ATPase, H+ transporting, lysosomal | | | | 1.30 | -1.28 | 1.14 |
| 38126_at | biglycan | | | | | 1.75 | -1.61 |
| 39407_at | bone morphogenetic protein 1 | | | -1.20 | | -1.55 | |
| 31399_at | bone morphogenetic protein 10 | 1.44 | 1.45 | -1.31 | -1.77 | | |
| 1113_at | bone morphogenetic protein 2A | -1.12 | 2.63 | | | | 1.29 |
| 1831_at | bone morphogenetic protein 5 | -1.43 | 1.39 | 1.40 | | | |
| 1733_at | bone morphogenetic protein 6 precursor | | -1.37 | -1.17 | -1.64 | -1.27 | -1.1 |
| 34500_at | calcium binding protein 1 (calbrain) | | 2.31 | | | 1.21 | |
| 31670_s_at | calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma | | 1.17 | | 1.57 | -1.28 | 1.60 |
| 1751_g_at | calreticulin | -4.03 | -1.60 | | 1.67 | | |
| 32067_at | cAMP responsive element modulator (CREM) | 1.39 | | -1.24 | | -1.50 | |
| 39241_at | carbonic anhydrase I | -2.68 | | 1.18 | | -1.69 | |
| 40095_at | carbonic anhydrase II | -1.69 | | | | | |
| 40163_r_at | cartilage oligomeric matrix protein precursor | 2.36 | 5.61 | | | | |
| 128_at | cathepsin k | 1.18 | | | 1.35 | -2.33 | |
| 129_g_at | cathepsin k | 1.20 | -1.54 | | 1.17 | -1.28 | |
| 38466_at | cathepsin k | 1.27 | | | 1.40 | | -1.19 |
| 40718_at | cathepsin w | -1.31 | | -1.54 | | 2.05 | |
| 32833_at | CDC-like kinase 1 | 1.63 | | | | | |
| 646_s_at | CDC-like kinase 2 isoform hclk2/139 | 1.19 | | | 1.86 | | |
| 38112_g_at | chondroitin sulfate proteoglycan 2 (versican) | | -2.16 | | 1.51 | | -1.68 |
| 32642_at | chondroitin sulfate proteoglycan 3 (neurocan) | | | | | -1.49 | |
| 31493_s_at | chorionic somatomammotropin hormone 1 | | | | | -1.59 | |
| 40714_at | chymotrypsin C (caldecrin) | | | | | 1.39 | 3.22 |

## TABLE 36

## Multi-Organ Gene Expression Profiling of Salmon Calcitonin

| GeneChip® expression probe set identifier | Coding Gene | bone | kidney | liver | muscle | pituitary | trachea |
|---|---|---|---|---|---|---|---|
| 35474_s_at | Collagen type I and PDGFB fusion transcript | | | | -7.30 | | -3.35 |
| 598_at | collagen type II alpha-1 | -1.38 | | 1.69 | -1.27 | 2.77 | -3.02 |
| 32488_at | collagen type III alpha 1 | -1.41 | -1.59 | -1.53 | -3.20 | -1.89 | -1.35 |
| 38952_s_at | collagen type IV alpha-2 | 1.23 | | | | -1.73 | |
| 35379_at | collagen type IX alpha1 | -2.22 | -3.28 | | | | |
| 38722_at | collagen type VI alpha-1 | | -3.38 | -1.13 | -1.42 | | |
| 34802_at | collagen type VI alpha-2 (AA 570-998) | -1.37 | | -1.10 | -1.39 | | -1.28 |
| 37892_at | collagen type XI alpha-1 | 1.24 | | | | -2.46 | -1.51 |
| 1026_s_at | collagen type XI alpha2 | -1.20 | -1.32 | | | 1.15 | -2.20 |
| 1027_at | collagen type XI alpha2 | 1.11 | | -1.25 | | | 1.37 |
| 32305_at | collagen, type I, alpha 2 | | -1.45 | | | | -1.54 |
| 39333_at | collagen, type IV, alpha 1 | | | | | | -1.49 |
| 39925_at | collagen, type IX, alpha 2 | | | | | -2.38 | -1.36 |
| 38420_at | collagen, type V, alpha 2 | | -1.29 | -1.18 | -1.11 | | -1.10 |
| 41351_at | collagen, type VI, alpha 1 | | -2.29 | | -1.27 | -1.50 | |
| 41350_at | collagen, type VI, alpha 1 precursor | | | | | | -3.55 |
| 35168_f_at | collagen, type XVI, alpha 1 | | | | | | -1.59 |
| 35169_at | collagen, type XVI, alpha 1 | | | | | | -1.18 |
| 39632_at | collagenase 3 (matrix metalloproteinase 13) | 1.20 | | | | | |
| 36638_at | connective tissue growth factor | | | | | -2.11 | |
| 40697_at | cyclin A2 | -1.60 | | | | | |
| 34736_at | cyclin B1 | -2.83 | | | | | |
| 36650_at | cyclin D2 | 1.21 | | | | | |
| 35249_at | cyclin E2 | -2.95 | | | | | |
| 1206_at | cyclin-dependent kinase 5 | 1.56 | | | | | -1.54 |
| 799_at | cyclin-dependent kinase 5, regulatory subunit 1 (p35) | 1.32 | | | | | |
| 41546_at | cyclin-dependent kinase 6 | 1.15 | 1.52 | | | 1.34 | |
| 2031_s_at | cyclin-dependent kinase inhibitor 1A (p21, Cip1) | 1.95 | | | | | |
| 35816_at | cystatin B (stefin B) | 1.57 | | | | | |
| 806_at | cytokine-inducible kinase | 1.20 | | | | 1.35 | |
| 40049_at | death-associated protein kinase 1 | | | | | -1.47 | -1.29 |
| 33903_at | death-associated protein kinase 3 | -1.22 | | | | | |
| 34029_at | dentin matrix acidic phosphoprotein 1 (DMP1) | 1.65 | | | | | |
| 40186_at | dual specificity phosphatase 9 | | | | | | 1.59 |
| 37996_s_at | dystrophia myotonica-protein kinase | | 1.25 | | | | -1.50 |
| 342_at | ectonucleotide Pyrophosphatase/ Phosphodiesterase 1; | 1.45 | | | | | |
| 343_s_at | ectonucleotide pyrophosphatase/ phosphodiesterase 1; | 1.11 | | | -1.42 | | |
| 33602_at | endothelial differentiation, G protein coupled receptor 6 precursor | | 1.15 | | | 2.24 | -1.66 |
| 1442_at | estrogen receptor | 1.47 | 1.23 | | | 1.60 | |
| 33670_at | estrogen receptor | 1.30 | | | | | |
| 1487_at | estrogen receptor-related protein | 1.11 | | | -1.52 | | 1.24 |

## TABLE 36
## Multi-Organ Gene Expression Profiling of Salmon Calcitonin

| GeneChip® expression probe set identifier | Coding Gene | bone | kidney | liver | muscle | pituitary | trachea |
|---|---|---|---|---|---|---|---|
| 38882_r_at | estrogen-responsive B box protein (EBBP) | 1.22 | | -1.51 | | | |
| 39945_at | fibroblast activation protein | -1.27 | | | | -1.48 | -1.32 |
| 996_at | fibroblast growth factor 1 (acidic) | | 1.17 | | -1.41 | | |
| 41586_at | fibroblast growth factor 18 | | 2.06 | | | | |
| 1730_s_at | fibroblast growth factor 4 | | 1.55 | | | | 1.46 |
| 424_s_at | fibroblast growth factor receptor. | -1.17 | -1.59 | | | | |
| 40131_at | follistatin-like 1 | -1.31 | | | | | |
| 40132_g_at | follistatin-like 1 | | | | | -1.22 | 1.15 |
| 33510_s_at | glutamate receptor, metabotropic 1 | 1.26 | | | -1.31 | | |
| 33269_at | GPI1 N-acetylglucosaminyl transferase component Gpi1 | 1.24 | | | | | |
| 1401_g_at | granulocyte-macrophage colony-stimulating factor (CSF1) | -3.07 | 2.24 | | | | |
| 1911_s_at | growth arrest and DNA-damage-inducible, alpha | | 1.84 | | -3.84 | | 1.24 |
| 37615_at | growth factor receptor-bound protein 10 | 1.21 | | | | -1.61 | |
| 32845_at | heparan sulfate proteoglycan 2 (perlecan) | | | 1.27 | | | -1.11 |
| 32778_at | inositol 1,4,5-triphosphate receptor, type 1 | | 1.75 | | | -2.57 | 1.20 |
| 32779_s_at | inositol 1,4,5-triphosphate receptor, type 1 | | | 1.21 | 2.02 | | |
| 756_at | inositol 1,4,5-triphosphate receptor, type 2 | | | | | | 1.24 |
| 34209_at | inositol 1,4,5-trisphosphate 3-kinase isoenzyme | | 2.29 | | 1.42 | -1.36 | 1.75 |
| 33506_at | inositol polyphosphate 4-phosphatase type I-beta | 1.12 | 1.66 | 2.09 | | 1.27 | |
| 172_at | inositol polyphosphate-5-phosphatase, | | | -1.22 | -1.15 | | |
| 32697_at | inositol(myo)-1(or 4)-monophosphatase 1 | -1.36 | -2.70 | | | 1.61 | |
| 36496_at | inositol(myo)-1(or 4)-monophosphatase 2 | | | | | | 1.13 |
| 2079_s_at | insulin-like growth factor (IGF-II) | | | | -1.32 | 1.15 | -1.31 |
| 36782_s_at | insulin-like growth factor 2 (somatomedin A) | | | | | | -1.69 |
| 1232_s_at | insulin-like growth factor binding protein | -1.31 | | | -1.53 | | |
| 40422_at | insulin-like growth factor binding protein 2 | | | | | -2.97 | -1.16 |
| 1586_at | insulin-like growth factor binding protein 3 | 1.45 | | -1.16 | 1.70 | | |
| 37319_at | insulin-like growth factor binding protein 3 | 2.17 | | | 1.58 | | -1.52 |
| 41420_at | insulin-like growth factor binding protein 5 | | 1.15 | | -2.66 | | |
| 1741_s_at | insulin-like growth factor binding protein-2 | -2.49 | | | | -2.17 | -1.22 |
| 1464_at | insulin-like growth factor II precursor | 1.18 | 1.10 | | | -1.26 | |

## TABLE 36
## Multi-Organ Gene Expression Profiling of Salmon Calcitonin

| GeneChip® expression probe set identifier | Coding Gene | bone | kidney | liver | muscle | pituitary | trachea |
|---|---|---|---|---|---|---|---|
| 1591_s_at | insulin-like growth factor II precursor | 1.41 | | | | | -2.80 |
| 33082_at | integrin alpha 10 subunit | 1.33 | | | -2.32 | -1.18 | |
| 1100_at | interleukin-1 receptor-associated kinase | | | | 1.39 | -1.48 | |
| 2005_s_at | Janus kinase 3 | | | | -1.51 | | 1.57 |
| 40060_r_at | LIM protein (similar to rat protein kinase C-binding enigma) | 1.44 | | | | -1.68 | -1.31 |
| 36811_at | lysyl oxidase-like protein | -1.44 | 1.14 | | 1.30 | -1.19 | |
| 1433_g_at | MAD, mothers against decapentaplegic homolog 3 | 1.14 | -1.13 | | -1.61 | -1.65 | -1.69 |
| 34655_at | MAGUKs (membrane-associated guanylate kinase homologues | 1.23 | | | | | |
| 35652_g_at | MAP kinase kinase kinase (MTK1) | 1.14 | | | | | |
| 33246_at | MAPK13: mitogen-activated protein kinase 13 | | | -1.24 | -1.13 | -1.91 | 1.65 |
| 41280_r_at | MAPK8IP1: mitogen-activated protein kinase 8 interacting protein 1 | -1.31 | 1.92 | | | 1.58 | |
| 2004_at | MEK kinase | | | | 1.13 | -1.62 | 1.16 |
| 1509_at | metalloproteinase | -1.42 | | -1.11 | | -1.23 | -1.18 |
| 976_s_at | mitogen-activated protein kinase 1 | -1.61 | | | | | |
| 34006_s_at | mitogen-activated protein kinase 8 | 1.32 | | | | | |
| 1844_s_at | mitogen-activated protein kinase kinase 1 | | | | | -1.60 | 1.15 |
| 35694_at | mitogen-activated protein kinase kinase kinase kinase 4 | | | | 1.26 | | |
| 1469_at | mitogen-activated protein kinase-activated protein kinase 2 | | 1.13 | -1.30 | | | 1.16 |
| 1637_at | mitogen-activated protein kinase-activated protein kinase 3 | | | | 1.11 | | 1.34 |
| 37565_at | MMD: monocyte to macrophage differentiation-associated | 1.28 | -2.48 | | | | -1.28 |
| 38307_at | neurochondrin, | | | 2.80 | | -1.39 | |
| 39144_at | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 | | 2.72 | | 1.42 | | -1.70 |
| 41202_s_at | OS-4 protein (OS-4) | 1.24 | | | | -1.72 | |
| 1451_s_at | OSF-2os osteoblast specific factor-2 (periostin) | -1.65 | | | | -2.06 | 1.56 |
| 467_at | osteoclast stimulating factor (OSF) | -1.23 | -1.50 | -1.58 | | -4.12 | |
| 33814_at | PAK4 | 1.16 | | | -1.33 | 1.11 | |
| 38757_at | PDGF associated protein. | -1.89 | | | | -1.15 | 1.20 |
| 146_at | phosphatidylinositol 4-kinase, catalytic, beta polypeptide | | | | 1.19 | | 1.23 |
| 34496_at | phosphatidylinositol glycan, class L | | 2.34 | 1.34 | 1.51 | | |
| 34169_s_at | phosphatidylinositol polyphosphate 5-phosphotase, isoform b | | | | | -1.33 | 1.49 |
| 37412_at | phosphatidylinositol-4-phosphate 5-kinase isoform C (-1) | -1.87 | | -1.31 | | | |
| 37253_at | phosphatidylinositol-4-phosphate 5-kinase, type I, beta | | 1.17 | | -1.13 | | 1.11 |

TABLE 36

Multi-Organ Gene Expression Profiling of Salmon Calcitonin

| GeneChip® expression probe set identifier | Coding Gene | bone | kidney | liver | muscle | pituitary | trachea |
|---|---|---|---|---|---|---|---|
| 35741_at | phosphatidylinositol-4-phosphate 5-kinase, type II, beta | | | | -1.18 | | -1.18 |
| 751_at | phosphatidylinositol-glycan-class C (PIG-C) | 1.14 | 1.19 | | | -1.22 | |
| 666_at | phosphodiesterase 4A, cAMP-specific | 1.33 | | -1.32 | | -1.18 | |
| 38526_at | phosphodiesterase 4D, cAMP-specific (dunce (Drosophila)-homolog phosphodiesterase E3) | 1.30 | 1.15 | | | | 3.51 |
| 38921_at | phosphodiesterase IB, calmodulin-dependent | 1.52 | | | | 1.42 | 1.12 |
| 31699_at | phosphoinositide-3-kinase | 1.56 | | -1.56 | | | |
| 36287_at | phosphoinositide-3-kinase, catalytic, gamma polypeptide | 1.31 | | | | | |
| 35665_at | phosphoinositide-3-kinase, class 3 | | | | | -1.11 | 1.21 |
| 364_s_at | phospholipase C b3 | 1.22 | | | | | |
| 901_g_at | phospholipase C, beta 4 | -1.20 | | 1.41 | | -1.55 | |
| 1293_s_at | phospholipase D | -1.26 | | | | | |
| 38023_at | phosphotidylinositol transfer protein | | 2.25 | | 1.33 | 1.55 | 1.71 |
| 38269_at | PKD2 Protein kinase D2 | | | 1.34 | | | |
| 32306_g_at | preprocollagen type I alpha-2 | 1.19 | | -1.38 | | -1.75 | -1.31 |
| 35473_at | preprocollagen type I alpha1. | -2.72 | -1.37 | | -3.94 | | -2.70 |
| 32307_s_at | procollagen | 1.13 | | -1.26 | -2.44 | -1.56 | -1.82 |
| 37605_at | procollagen alpha 1 type II | | | | -1.84 | | -1.61 |
| 36184_at | procollagen-lysine 5-dioxygenase | | 2.52 | | -2.15 | | -1.30 |
| 37037_at | procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide I | | | 1.87 | 1.46 | -1.67 | 1.29 |
| 37633_s_at | progestagen-associated endometrial protein (placental protein 14, pregnancy-associated endometrial alpha-2-globulin, alpha uterine protein) | | 2.00 | | | | |
| 36109_at | prolidase (imidodipeptidase) PEPD: | -2.55 | | | | -2.05 | |
| 1884_s_at | proliferating cell nuclear antigen | -1.85 | | | | | |
| 36666_at | prolyl 4-hydroxylase beta | 1.95 | | | 1.37 | 2.08 | |
| 718_at | protease, serine, 11 (IGF binding) | -1.30 | | | | -1.81 | -1.30 |
| 719_g_at | protease, serine, 11 (IGF binding) | -1.43 | | | | -1.97 | -1.27 |
| 385_at | proteasome (prosome, macropain) subunit, beta type, 10 | 1.36 | | -1.29 | | | |
| 37431_at | protein inhibitor of activated STAT X | | | | | -1.23 | 1.28 |
| 39183_at | protein kinase 1 PCTAIRE | -1.17 | | | | | |
| 39711_at | protein kinase C substrate 80K-H | | | | | | 1.31 |
| 1437_at | protein kinase C, alpha | | | -2.06 | | | 1.82 |
| 36359_at | protein kinase, cAMP-dependent, catalytic, gamma | 1.39 | | 1.14 | -1.49 | 1.30 | 1.13 |
| 1091_at | protein kinase, cAMP-dependent, regulatory, type I, beta | 1.65 | -1.80 | | 2.06 | | |
| 116_at | protein kinase, cAMP-dependent, regulatory, type II, alpha | 1.28 | | | -1.18 | | |
| 33633_at | purinergic receptor P2Y, G-protein coupled, 11 | 1.90 | -1.82 | | | | |

## TABLE 36

## Multi-Organ Gene Expression Profiling of Salmon Calcitonin

| GeneChip® expression probe set identifier | Coding Gene | bone | kidney | liver | muscle | pituitary | trachea |
|---|---|---|---|---|---|---|---|
| 32737_at | RAC2 Ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2) | 1.16 | | | | | 1.22 |
| 1007_s_at | receptor tyrosine kinase DDR | 1.21 | | | | | |
| 1048_at | retinoid X receptor-gamma | 1.47 | | | | 1.47 | |
| 41404_at | ribosomal protein S6 kinase | -1.67 | -1.40 | | -1.83 | | -1.40 |
| 865_at | ribosomal protein S6 kinase, 90kD, polypeptide 3 | | -1.42 | | | | 1.27 |
| 32290_at | SCAMP1: secretory carrier membrane protein 1 (vesicular transport) | 2.50 | | | | -1.27 | -1.39 |
| 34342_s_at | secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1) | | 1.15 | | | | -3.01 |
| 39166_s_at | serine (or cysteine) proteinase inhibitor, clade H (heat shock protein 47), member 2 | | -2.82 | 1.56 | 2.04 | | -1.29 |
| 36217_at | serine/threonine kinase 38 | 1.54 | | | | | -1.59 |
| 1223_at | serine/threonine protein kinase | | | 2.42 | | | |
| 32447_at | SF-1; Steroidogenic factor-1 | 8.76 | | 1.59 | | 1.27 | -2.01 |
| 33338_at | signal transducer and activator of transcription 1 | | | -1.14 | 1.15 | -2.11 | -1.93 |
| 1244_at | signal transducer and activator of transcription 2, 113kD | | | | | | 1.57 |
| 40458_at | signal transducer and activator of transcription 5A | | | | | 1.14 | 1.39 |
| 506_s_at | signal transducer and activator of transcription 5A | | | | 1.32 | 2.60 | |
| 41222_at | signal transducer and activator of transcription 6 (STAT6) | 1.44 | | | 1.14 | -1.46 | |
| 1950_s_at | Smad 3 | | | | -2.44 | | -1.16 |
| 38889_at | Smad anchor for receptor activation, isoform 1 | | 1.28 | | | -1.14 | -1.51 |
| 1013_at | Smad5 | | | | | -2.62 | 1.22 |
| 1955_s_at | SMAD6 (inhibits BMP/Smad1 (MADH1) | 1.19 | | | | -1.37 | |
| 37718_at | SNF-1 related kinase | 1.49 | | -1.13 | 1.18 | | |
| 35883_at | Spi-B transcription factor (SPI1/PU.1 related) | | 3.76 | | | -2.96 | 1.15 |
| 472_at | Stat5b (stat5b) | -1.42 | -1.28 | | | -1.83 | -2.50 |
| 38669_at | Ste20-related serine/threonine kinase | 1.24 | | | | -1.78 | |
| 38374_at | TEIG; TGFβ inducible early growth response | 1.18 | | | | -1.79 | |
| 224_at | TGFβ inducible early growth response; TIEG | 1.26 | | | | -2.69 | |
| 36940_at | TGFβ1-induced anti-apoptotic factor 1 | 1.22 | | | 1.28 | -1.38 | |
| 32217_at | TGF-beta induced apoptosis protein 12 | 1.40 | 1.55 | | 1.12 | | |
| 41445_at | TGF-beta precursor | 1.14 | 1.11 | | | | |
| 1890_at | TGF-beta superfamily protein | 1.74 | 1.85 | 1.12 | | 1.38 | |
| 40631_at | Tob | -1.14 | | | 1.28 | 2.09 | |
| 32219_at | tousled-like kinase 1 | | -1.16 | | | | |

TABLE 36

Multi-Organ Gene Expression Profiling of Salmon Calcitonin

| GeneChip® expression probe set identifier | Coding Gene | bone | kidney | liver | muscle | pituitary | trachea |
|---|---|---|---|---|---|---|---|
| 1897_at | transforming growth factor, beta receptor III (betaglycan, 300kD) | | 1.18 | | | | 1.12 |
| 1735_g_at | transforming growth factor-beta 3 | | -1.15 | | -4.45 | -1.39 | -2.23 |
| 1767_s_at | transforming growth factor-beta 3 (TGF-beta 3) | -1.71 | 1.41 | | -1.71 | | |
| 40581_at | TRIO: triple functional domain (PTPRF interacting) | 1.65 | 1.62 | | 1.34 | | -1.42 |
| 32272_at | tubulin alpha | -1.20 | | | 1.18 | | |
| 330_s_at | tubulin alpha 1 | -1.80 | | 1.23 | -1.20 | -1.19 | |
| 40567_at | tubulin alpha 3 | -1.39 | | -1.18 | | | -1.10 |
| 685_f_at | tubulin alpha isotype H2-alpha | -4.36 | | 1.32 | | 2.13 | |
| 151_s_at | tubulin beta | -1.40 | -1.14 | 1.16 | 1.22 | 1.16 | |
| 33678_i_at | tubulin beta 2 | -1.15 | | | 1.75 | | |
| 33679_f_at | tubulin beta 2 | -1.31 | | | 1.45 | | |
| 709_at | tubulin beta 3 | -1.18 | | | | -1.35 | 1.20 |
| 471_f_at | tubulin beta 4 | -1.38 | | | 1.50 | | |
| 39399_at | tubulin beta, cofactor D | -1.85 | | | | -4.69 | |
| 32098_at | type VI collagen alpha 2 chain precursor | | | | | | -3.79 |
| 1651_at | ubiquitin carrier protein E2-C | -3.74 | | | | | |
| 1953_at | vascular endothelial growth factor | 1.40 | | | | | |
| 36101_s_at | vascular endothelial growth factor | 1.45 | | | | | |
| 37268_at | vascular endothelial growth factor B | | | | -1.58 | | |
| 36140_at | Y box binding protein-1 | 2.30 | 1.86 | | 2.36 | -2.72 | |

[00177] In addition, the effect of PTS893 was assessed in bone.

TABLE 37

Gene-Profiling Analysis of Salmon Calcitonin and PTS893 in Bone

| GeneChip® Expression Probe Set Identifier | Coding Gene | Fold Increase Salmon Calcitonin | Fold Increase PTS893 |
|---|---|---|---|
| 38909_at | 25-hydroxyvitamin D3 1-alpha-hydroxylase | | -1.14 |
| 32714_s_at | activin A receptor type II-like 1 | -1.62 | |
| 35915_at | activin beta-C chain. | -1.21 | |
| 39279_at | activin type II receptor | | 1.24 |
| 39383_at | adenylate cyclase 6, isoform a | | -1.22 |
| 38965_at | aggrecan 1 | | 2.03 |
| 39206_s_at | aggrecan 1 | | 1.41 |
| 36621_at | alpha-2-HS-glycoprotein | 1.33 | |
| 34589_f_at | Amelogenin | 1.10 | -3.10 |
| 39326_at | ATPase H(+) vacuolar | -1.57 | -1.19 |
| 38814_at | ATPase H(+) vacuolar | 1.22 | |
| 33741_at | ATPase, H+ transport, lysosomal | 1.23 | |
| 33033_at | ATPase, H+ transporting, lysosomal | -1.29 | -1.17 |
| 40328_at | bHLH transcription factor | | 2.57 |
| 39407_at | bone morphogenetic protein 1 | | 1.16 |
| 31399_at | bone morphogenetic protein 10 | 1.44 | 1.20 |

TABLE 37

Gene-Profiling Analysis of Salmon Calcitonin and PTS893 in Bone

| GeneChip® Expression Probe Set Identifier | Coding Gene | Fold Increase Salmon Calcitonin | Fold Increase PTS893 |
|---|---|---|---|
| 1113_at | bone morphogenetic protein 2A | -1.12 | -1.13 |
| 40367_at | bone morphogenetic protein 2A | | -1.18 |
| 1114_at | bone morphogenetic protein 2B or BMP4 | | -1.70 |
| 1831_at | bone morphogenetic protein 5 | -1.43 | -1.60 |
| 1733_at | bone morphogenetic protein 6 precursor | | 1.27 |
| 40333_at | bone morphogenetic protein-4 (hBMP-4) | | -1.42 |
| 34847_s_at | calcium/calmodulin-dependent protein kinase (CaM kinase) II beta | | 1.13 |
| 33935_at | calcyclin binding protein | | 1.41 |
| 1751_g_at | Calreticulin | -4.03 | |
| 32067_at | cAMP responsive element modulator (CREM) | 1.39 | 2.75 |
| 39241_at | carbonic anhydrase I | -2.68 | |
| 40095_at | carbonic anhydrase II | -1.69 | |
| 40163_r_at | cartilage oligomeric matrix protein precursor | 2.36 | |
| 128_at | cathepsin k | 1.18 | |
| 129_g_at | cathepsin k | 1.20 | |
| 38466_at | cathepsin k | 1.27 | |
| 40718_at | cathepsin w | -1.31 | |
| 32833_at | CDC-like kinase 1 | 1.63 | |
| 646_s_at | CDC-like kinase 2 isoform hclk2/139 | 1.19 | |
| 34763_at | chondroitin sulfate proteoglycan 6 | | -1.18 |
| 598_at | collagen type II alpha-1 | -1.38 | -1.19 |
| 32488_at | collagen type III alpha 1 | -1.41 | |
| 38952_s_at | collagen type IV alpha-2 | 1.23 | 1.44 |
| 35379_at | collagen type IX alpha1 | -2.22 | |
| 34802_at | collagen type VI alpha-2 (AA 570-998) | -1.37 | |
| 38566_at | collagen type X alpha-1 | | 1.67 |
| 37892_at | collagen type XI alpha-1 | 1.24 | 1.18 |
| 1026_s_at | collagen type XI alpha2 | -1.20 | |
| 1027_at | collagen type XI alpha2 | 1.11 | |
| 39632_at | collagenase 3 (matrix metalloproteinase 13) | 1.20 | |
| 36638_at | connective tissue growth factor. | | -1.32 |
| 1943_at | cyclin A | | -1.74 |
| 40697_at | cyclin A2 | -1.60 | -1.39 |
| 34736_at | cyclin B1 | -2.83 | |
| 39251_at | cyclin C | | -2.03 |
| 1983_at | cyclin D2 | | -1.28 |
| 36650_at | cyclin D2 | 1.21 | |
| 35249_at | cyclin E2 | -2.95 | |
| 1649_at | cyclin G1 interacting protein | | 1.31 |
| 1913_at | cyclin G2 | | -1.29 |
| 160024_at | cyclin-dependent kinase (CDC2-like) 10 PISSLRE | | 1.53 |
| 1942_s_at | cyclin-dependent kinase 4 | | -1.22 |
| 1206_at | cyclin-dependent kinase 5 | 1.56 | |
| 40549_at | cyclin-dependent kinase 5 | | -1.40 |
| 799_at | cyclin-dependent kinase 5, regulatory subunit 1 (p35) | 1.32 | |
| 41546_at | cyclin-dependent kinase 6 | 1.15 | |
| 2031_s_at | cyclin-dependent kinase inhibitor 1A (p21, Cip1) | 1.95 | |
| 1787_at | cyclin-dependent kinase inhibitor 1C | | 1.18 |
| 38673_s_at | cyclin-dependent kinase inhibitor 1C | | 1.13 |

## TABLE 37

## Gene-Profiling Analysis of Salmon Calcitonin and PTS893 in Bone

| GeneChip® Expression Probe Set Identifier | Coding Gene | Fold Increase Salmon Calcitonin | Fold Increase PTS893 |
|---|---|---|---|
| 39545_at | cyclin-dependent kinase inhibitor 1C | | 1.24 |
| 1797_at | cyclin-dependent kinase inhibitor 2D (p19, inhibits CDK4) | | -1.21 |
| 35816_at | cystatin B (stefin B) | 1.57 | |
| 806_at | cytokine-inducible kinase | 1.20 | |
| 40049_at | death-associated protein kinase 1 | | -1.30 |
| 33903_at | death-associated protein kinase 3 | -1.22 | -7.73 |
| 34029_at | dentin matrix acidic phosphoprotein 1 (DMP1) | 1.65 | |
| 38059_g_at | dermatopontin | | 1.72 |
| 343_s_at | ectonucleotide pyrophosphatase/ phosphodiesterase 1 | 1.11 | |
| 342_at | ectonucleotide Pyrophosphatase/ Phosphodiesterase 1 | 1.45 | |
| 1442_at | estrogen receptor | 1.47 | |
| 33670_at | estrogen receptor | 1.30 | |
| 1487_at | estrogen receptor-related protein | 1.11 | |
| 38882_r_at | estrogen-responsive B box protein (EBBP) | 1.22 | |
| 38902_r_at | estrogen-responsive B box protein (EBBP) | | 1.23 |
| 39945_at | fibroblast activation protein | -1.27 | |
| 424_s_at | fibroblast growth factor receptor. | -1.17 | |
| 466_at | general transcription factor II, | | 1.34 |
| 1102_s_at | glucocorticoid receptor alpha | | 1.43 |
| 33510_s_at | glutamate receptor, metabotropic 1 | 1.26 | 1.23 |
| 33269_at | GPI1 N-acetylglucosaminyl transferase component Gpi1 | 1.24 | 1.21 |
| 41476_at | G-protein alpha subunit 11 | | 1.24 |
| 1401_g_at | granulocyte-macrophage colony-stimulating factor (CSF1) | -3.07 | -2.57 |
| 1911_s_at | growth arrest and DNA-damage-inducible protein (gadd45) | | 2.87 |
| 888_s_at | growth differentiation factor 1 | | -1.43 |
| 37615_at | growth factor receptor-bound protein 10 | 1.21 | |
| 33929_at | heparan sulfate proteoglycan (glypican). | | 2.00 |
| 39757_at | heparan sulfate proteoglycan core protein | | 1.10 |
| 755_at | inositol 1,4,5-trisphosphate receptor type 1 | | 1.27 |
| 33506_at | inositol polyphosphate 4-phosphatase type I-beta | 1.12 | -1.24 |
| 33290_at | inositol polyphosphate 5-phosphatase (5ptase) | | -1.20 |
| 32697_at | inositol(myo)-1(or 4)-monophosphatase 1 | -1.36 | |
| 1975_s_at | insulin-like growth factor 1 | | -1.41 |
| 1501_at | insulin-like growth factor 1 (somatomedin C) | | -1.12 |
| 1232_s_at | insulin-like growth factor binding protein | -1.31 | |
| 40422_at | insulin-like growth factor binding protein 2 | | -1.27 |
| 1586_at | insulin-like growth factor binding protein 3 | 1.45 | |
| 37319_at | insulin-like growth factor binding protein 3 | 2.17 | |
| 1737_s_at | insulin-like growth factor binding protein 4 | | 1.13 |
| 41420_at | insulin-like growth factor binding protein 5 | | 1.18 |
| 1396_at | insulin-like growth factor binding protein 5 | | 1.62 |
| 1678_g_at | insulin-like growth factor binding protein 5 | | 1.44 |
| 38650_at | insulin-like growth factor binding protein 5 | | 1.53 |
| 1741_s_at | insulin-like growth factor binding protein-2 | -2.49 | -2.11 |
| 1464_at | insulin-like growth factor II precursor | 1.18 | |
| 1591_s_at | insulin-like growth factor II precursor | 1.41 | 1.31 |
| 39781_at | insulin-like growth factor-binding protein 4 | | 1.16 |
| 33082_at | integrin alpha 10 subunit | 1.33 | |
| 35131_at | integrin-binding sialoprotein (bone sialoprotein, bone sialoprotein II) | | 1.15 |

## TABLE 37

## Gene-Profiling Analysis of Salmon Calcitonin and PTS893 in Bone

| GeneChip® Expression Probe Set Identifier | Coding Gene | Fold Increase Salmon Calcitonin | Fold Increase PTS893 |
|---|---|---|---|
| 40060_r_at | LIM protein (similar to rat protein kinase C-binding enigma) | 1.44 | 1.32 |
| 36184_at | lysyl hydroxylase (PLOD) procollagen-lysine, 2-oxoglutarate 5 dioxygenase | | -1.40 |
| 34795_at | lysyl hydroxylase isoform 2 (PLOD2) | | 1.49 |
| 36811_at | lysyl oxidase-like protein | -1.44 | |
| 1433_g_at | MAD, mothers against decapentaplegic homolog 3 | 1.14 | 1.73 |
| 34655_at | MAGUKs (membrane-associated guanylate kinase homologues | 1.23 | |
| 36179_at | MAP kinase activated protein kinase 2 | | 1.18 |
| 35652_g_at | MAP kinase kinase kinase (MTK1) | 1.14 | |
| 41279_f_at | MAPK8IP1 Mitogen-activated protein kinase 8 interacting protein 1 | | 1.25 |
| 41280_r_at | MAPK8IP1: mitogen-activated protein kinase 8 interacting protein 1 | -1.31 | -1.31 |
| 1509_at | Metalloproteinase | -1.42 | |
| 976_s_at | mitogen-activated protein kinase 1 | -1.61 | 1.12 |
| 34006_s_at | mitogen-activated protein kinase 8 | 1.32 | |
| 1439_s_at | mitogen-activated protein kinase-activated protein kinase 2 | | 1.78 |
| 37565_at | MMD: monocyte to macrophage differentiation-associated | 1.28 | 1.30 |
| 38369_at | myeloid differentiation primary response gene (88) | | -1.10 |
| 1052_s_at | NF-IL6-beta protein | | 1.30 |
| 36472_at | N-myc and STAT interacter- | | -1.35 |
| 38354_at | nuclear factor NF-IL6 (AA 1-345) | | 1.92 |
| 33106_at | nuclear orphan receptor LXR-alpha nuclear receptor subfamily 1, group H, member 3 | | 3.29 |
| 33381_at | nuclear receptor co-activator | | 1.11 |
| 279_at | nuclear receptor subfamily 4, group A, member 1 | | 2.30 |
| 280_g_at | nuclear receptor subfamily 4, group A, member 1 | | 3.08 |
| 37623_at | nuclear receptor subfamily 4, group A, member 2 Member of the steroid/thyroid hormone receptor family | | 27.72 |
| 547_s_at | nuclear receptor subfamily 4, group A, member 2 Member of the steroid/thyroid hormone receptor family | | 26.77 |
| 190_at | nuclear receptor subfamily 4, group A, member 3 Member of steroid/thyroid receptor family of nuclear hormone receptors | | 5.45 |
| 41202_s_at | OS-4 protein (OS-4) | 1.24 | |
| 1451_s_at | OSF-2os osteoblast specific factor-2 (periostin) | -1.65 | |
| 38822_at | O-sialoglycoprotein endopeptidase | | 2.43 |
| 467_at | osteoclast stimulating factor (OSF | -1.23 | |
| 35107_at | osteoprotegerin ligand | | 3.33 |
| 33814_at | PAK4 protein | 1.16 | |
| 38757_at | PDGF associated protein. | -1.89 | |
| 40253_at | phosphatidylinositol 4-kinase (NPIK-C). | 1.77 | |
| 37412_at | phosphatidylinositol-4-phosphate 5-kinase isoform C (-1) | -1.87 | |
| 751_at | phosphatidylinositol-glycan-class C (PIG-C) | 1.14 | -1.25 |
| 666_at | phosphodiesterase 4A, cAMP-specific | 1.33 | 1.30 |
| 38526_at | phosphodiesterase 4D, cAMP-specific | 1.30 | 3.53 |
| 38921_at | phosphodiesterase IB, calmodulin-dependent | 1.52 | |
| 38944_at | phosphodiesterase IB, calmodulin-dependent | | 1.17 |
| 32029_at | phosphoinositide dependent protein kinase-1 (3) | | 1.16 |
| 31699_at | phosphoinositide-3-kinase | 1.56 | 1.16 |
| 1085_s_at | phospholipase C | | -1.14 |
| 364_s_at | phospholipase C b3 | 1.22 | |

## TABLE 37

## Gene-Profiling Analysis of Salmon Calcitonin and PTS893 in Bone

| GeneChip® Expression Probe Set Identifier | Coding Gene | Fold Increase Salmon Calcitonin | Fold Increase PTS893 |
|---|---|---|---|
| 901_g_at | phospholipase C, beta 4 | -1.20 | |
| 1293_s_at | phospholipase D | -1.26 | |
| 32306_g_at | preprocollagen type I alpha-2 | 1.19 | |
| 35473_at | preprocollagen type I alpha1. | -2.72 | |
| 38951_at | PRKCQ Protein kinase C, theta | | 1.43 |
| 32307_s_at | procollagen | 1.13 | |
| 34494_at | procollagen I-N proteinase. | | 1.92 |
| 37605_at | procollagen type II alpha1 | | 1.91 |
| 36109_at | prolidase (imidodipeptidase) PEPD | -2.55 | |
| 1884_s_at | proliferating cell nuclear antigen | -1.85 | |
| 34390_at | prolyl 4-hydroxylase alpha (II) subunit | | 1.19 |
| 37037_at | prolyl 4-hydroxylase alpha subunit | | 1.20 |
| 36666_at | prolyl 4-hydroxylase beta | 1.95 | |
| 36533_at | prostacyclin synthase | | 1.20 |
| 718_at | protease, serine, 11 (IGF binding) | -1.30 | |
| 719_g_at | protease, serine, 11 (IGF binding) | -1.43 | |
| 385_at | proteasome (prosome, macropain) subunit, beta type, 10 | 1.36 | |
| 39183_at | protein kinase 1 PCTAIRE | -1.17 | |
| 37698_at | protein kinase A (PRKA) anchor protein 1 | | 1.29 |
| 39711_at | protein kinase C substrate 80K-H | | 1.13 |
| 39161_at | protein kinase Njmu-R1 | | 1.21 |
| 35348_at | protein kinase, AMP-activated, beta 1 non-catalytic subunit | | 2.10 |
| 36359_at | protein kinase, cAMP-dependent, catalytic, gamma | 1.39 | |
| 546_at | protein kinase, cAMP-dependent, catalytic, inhibitor alpha | | 1.14 |
| 227_g_at | protein kinase, cAMP-dependent, regulatory, type I, alpha | | 1.18 |
| 41768_at | protein kinase, cAMP-dependent, regulatory, type I, alpha | | 1.15 |
| 1091_at | protein kinase, cAMP-dependent, regulatory, type I, beta | 1.65 | |
| 116_at | protein kinase, cAMP-dependent, regulatory, type II, alpha | 1.28 | |
| 33633_at | purinergic receptor P2Y, G-protein coupled, 11 | 1.90 | |
| 32737_at | RAC2 Ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2) | 1.16 | |
| 40299_at | RE2 G-protein coupled receptor | | 1.24 |
| 35668_at | receptor (calcitonin) activity modifying protein 1 RAMP 1 | | 1.34 |
| 40696_at | receptor (TNFRSF)-interacting serine-threonine kinase 1 | | 1.12 |
| 1007_s_at | receptor tyrosine kinase DDR | 1.21 | |
| 37701_at | regulator of G-protein signalling 2, 24kD | | 2.06 |
| 1048_at | retinoid X receptor-gamma | 1.47 | 1.34 |
| 36217_at | serine/threonine kinase 38 | 1.54 | |
| 41544_at | serum-inducible kinase | | 1.16 |
| 32447_at | SF-1; Steroidogenic factor-1 | 8.76 | |
| 36487_at | short stature homeobox 2, | | -1.46 |
| 41222_at | signal transducer and activator of transcription 6 (STAT6) | 1.44 | |
| 1955_s_at | SMAD6 (inhibits BMP/Smad1 (MADH1) signalling) | 1.19 | |
| 37718_at | SNF-1 related kinase | 1.49 | 1.19 |
| 35883_at | Spi-B | | -2.80 |
| 1244_at | Stat2 | | -1.12 |
| 506_s_at | Stat5A | | 1.16 |
| 38994_at | STAT-induced STAT inhibitor-2 | | 1.25 |
| 38669_at | Ste20-related serine/threonine kinase | 1.24 | 1.65 |
| 37152_at | steroid hormone receptor superfamily | | 1.19 |

TABLE 37

Gene-Profiling Analysis of Salmon Calcitonin and PTS893 in Bone

| GeneChip® Expression Probe Set Identifier | Coding Gene | Fold Increase Salmon Calcitonin | Fold Increase PTS893 |
|---|---|---|---|
| 35844_at | syndecan 4 | | 1.37 |
| 38374_at | TEIG; TGFB inducible early growth response | 1.18 | |
| 38427_at | TEIG; TGFB inducible early growth response | | 1.38 |
| 32080_at | tetracycline transporter-like protein | | 1.41 |
| 224_at | TGFB inducible early growth response; TIEG | 1.26 | |
| 36940_at | TGFB1-induced anti-apoptotic factor 1 | 1.22 | 1.60 |
| 32217_at | TGF-beta induced apotosis protein 12 | 1.40 | |
| 41445_at | TGF-beta precursor | 1.14 | |
| 1890_at | TGF-beta superfamily protein | 1.74 | |
| 40631_at | Tob | -1.14 | 1.59 |
| 39358_at | transcriptional co-repressor nuclear receptor co-repressor 2 | | 1.42 |
| 1385_at | transforming growth factor induced protein | | 1.36 |
| 1830_s_at | transforming growth factor-beta | | 1.17 |
| 1767_s_at | transforming growth factor-beta 3 (TGF-beta 3) | -1.71 | -1.63 |
| 40581_at | TRIO: triple functional domain (PTPRF interacting) | 1.65 | 1.56 |
| 32272_at | tubulin alpha | -1.20 | |
| 685_f_at | tubulin alpha isotype H2-alpha | -4.36 | -1.79 |
| 330_s_at | tubulin alpha, 1, | -1.80 | -1.15 |
| 151_s_at | tubulin beta | -1.40 | |
| 39399_at | tubulin beta cofactor D | -1.85 | |
| 471_f_at | tubulin beta, 4 | -1.38 | |
| 40567_at | tubulin, alpha 3 | -1.39 | |
| 709_at | tubulin, beta 3 | -1.18 | |
| 33678_i_at | tubulin, beta, 2 | -1.15 | |
| 33679_f_at | tubulin, beta, 2 | -1.31 | |
| 1651_at | ubiquitin carrier protein E2-C | -3.74 | -1.22 |
| 32548_at | unactive progesterone receptor | | -1.33 |
| 1953_at | vascular endothelial growth factor | 1.40 | 1.20 |
| 36101_s_at | vascular endothelial growth factor | 1.45 | 1.44 |
| 36140_at | Y box binding protein-1 | 2.30 | 5.49 |

- numbers = fold down-regulated

+ numbers = fold up-regulated

[00178] *Real-time PCR*. Based on the DNA microarray data a set of transcripts was chosen for quantitative analysis by real time-PCR (RT-PCR).

[00179] Briefly, the method exploits the SyBr Green dye which intercalates into double stranded DNA. Accumulation of PCR products is detected directly by monitoring the increase in fluorescence of the SyBr Green dye. Reactions are characterised by the point in time during cycling when amplification of a PCR product is first detected rather than the amount of PCR product accumulated after a fixed number of cycles. The higher the starting copy number of nucleic acid target, the sooner a significant increase in fluorescence is observed.

[00180] From each RNA sample, cDNA was made using an Applied Biosystem kit (Applied Biosystems # N808-0234) following the recommendation of the manufacturer. The PCR mixture was prepared using the SyBr Green Universal PCR Master Mix (Applied Biosystems # 4309155) as follows: 5 µl cDNA template, 400 nM of each primer, 0.2 mM deoxynucleotide triphosphates, 1 mM MgCl2 and 0.5 U Taq DNA polymerase, 5 µl SyBr Green PCR buffer and RNase free water up to a final volume of 50 µl. The PCR was performed using the ABI Prism 7700 Sequence Detection System, after a step at 95°C for 10 min, the step-cycle program was performed for a total of 40 cycles as follows: 95°C for 30 s, 60°C for 1 min. A negative control was included: PCR reaction mixture with water in place of the cDNA sample.

[00181] The initial template concentration was determined based on the threshold cycle. The threshold cycle is the PCR cycle at which fluorescence is first detected above background and has been shown to be inversely proportional to the number of target copies present in the sample. Quantification was performed by calculating the unknown target concentration relative to an absolute standard and by normalizing to a validated endogenous control such as a housekeeping gene (β-actin). Results are presented as percentage of control, once the ratio between the numbers of molecule for the gene of interest divided by the number of molecule for beta-actin has been calculated.

[00182] Based on the DNA microarray data the following set of transcripts was chosen for quantitative analysis by RT-PCR: adhesion receptor CD44, angiopoietin, bone morphogenetic protein 5, carbonic anhydrase II, cartilage oligomeric matrix protein, cathepsin K, osteopontin, pre-pro-alpha-2 type I collagen, Spi-B and Y-box binding protein.

TABLE 38
Real Time PCR Results

| GeneChip® Expression Probe Set Identifier | Coding Gene | Treatment Effect Salmon Calcitonin (% respect to control) | Treatment Effect PTS893 (% respect to control) |
|---|---|---|---|
| 1372_at | adhesion receptor CD44 | No change | No change |
| 1929_at | angiopoietin-1 | No change | No change |
| 1831_at | bone morphogenetic protein 5 | +16 | +18 |
| 40095_at | carbonic anhydrase II | - 60 | No change |
| 40161_at | cartilage oligomeric matrix protein | +34.23 | No change |
| 128_at | cathepsin K | +67.2 | No change |
| 2092_s_at | osteopontin | No change | No change |
| 32306_g_at | pre-pro-alpha-2 type I collagen | +38 | +62 |
| 35883_at | Spi-B | -44 | -18 |
| 36140_at | Y-box binding protein (bone) | +14 | +26 |
| 36140_at | Y-box binding protein (kidney) | +15 | n.a. |
| 36140_at | Y-box binding protein (muscle) | -26 | n.a. |

n.a.: not applicable

[00183] RT-PCR confirmed in most of the cases the changes observed in the gene profiling analysis, as it was the case for bone morphogenetic protein 5, carbonic anhydrase II, cathepsin K, cartilage oligomeric matrix protein, pre-pro-alpha-2 type I collagen, Spi-B and Y-Box binding protein. No changes were however detected in the level of expression of adhesion receptor CD44, angiopoietin-1 and osteopontin.

[00184] *Analysis*. Calcitonin is known to exert an effect on the differentiation, survival and resorptive activity of osteoclasts, resulting in a decreased osteoclastic activity. Pondel M, *Intl. J. Exp. Pathol*. 81(6): 405-22 (2000). These effects could be reconstructed by multiorgan gene profiling (TABLE 39).

TABLE 39
Effects on Osteoclasts

| Function | Coding genes | Salmon Calcitonin | PTS893 |
|---|---|---|---|
| Osteoclast determination, survival and differentiation | PU.1 (SPI1) | B, K, P, T | B |
| | Granulocyte to macrophage colony-stimulating factor (CSF1) | B, K | B |
| | Monocyte to macrophage differentiation associate (MMD) | B, K, T | B |
| | Osteoclast stimulating factor 1 (Autocrine stimulation of osteoclast resorptive activity) | B, K, L, P | |
| Bone resorption by osteoclast | H+ ATP-ases | ALL | B |
| | Carbonic anhydrase I, II. | B, L, P | |
| | Cathepsin K | ALL | |
| | ODF/OPGL: osteoprotegerin ligand | | B |
| Osteoclast motility | Tubulins | ALL | |
| | PAK4 protein | B, M, P | |

Multiorgan gene expression profiling in salmon calcitonin treated animals. Organs where changes in expression were seen are displayed. B= bone; K= kidney; M= muscle; P= pituitary; L= liver; T= trachea.

[00185] PU.1 is involved in the initial stages of osteoclastogenesis. Tondravi MM *et al.*, *Nature* 386(6620): 81-4 (1997). CSF-1 is imperative for macrophage maturation; it binds to its receptor c-*fms* on early osteoclast precursors, providing signals required for their survival and proliferation. Teitelbaum SL, *Science* 289(5484):1504-1508 (2000).

[00186] Interestingly, PTS893 also regulates the genes implicated in osteoclast differentiation and survival, SPI1, CSF-1 and MMD. This osteoclast regulation has not been previously described.

[00187] Salmon calcitonin was shown to regulate the expression of the gene coding for osteoclast stimulating factor (OSF), which is an intracellular protein produced by osteoclasts that indirectly induces osteoclast formation and bone resorption. Reddy S *et al.*, *J. Cell Physiol*. 177 (4): 636-45 (1998). This would imply an autocrine effect of salmon calcitonin in the regulation of the osteoclast function, which is described here for the first time.

[00188] In addition, salmon calcitonin seems to exert a paracrine regulation of the osteoclast resorptive activity, through the regulation of cystatin expression in the osteoblast. Carbonic anhydrase I, II, $H^+$-ATPases and cathepsin K are the main effectors for dissolving

bone mineral and matrix degradation. Blair HC *et al., Biochem.* (2002). Regulation of tubulins and PAK4 genes can be related to the effect of calcitonin on osteoclast motility PAK 4. Zaidi M *et al., Bone* 30(5): 655-63 (2002); Jaffer ZM & Chernoff J, *Intl. J. Biochem. Cell Biol.* 34(7): 713-7 (2002).

[00189] These results show modulating effects of calcitonin on genes affecting the direct, autocrine, paracrine and endocrine regulation of the osteoblast function (TABLE 40). These data support the hypothesis that attributes a bone anabolic effect to calcitonin.

TABLE 40
Effects on Osteoblasts

| Function | Coding Gene | Salmon Calcitonin | PTS893 |
|---|---|---|---|
| Antagonists of cathepsins; antiresorptive activity | Cystatins | B | |
| Autocrine/paracrine regulation of osteoblast function | Alpha-2-HS-Glycoprotein | B, K, T | |
| | Bone Morphogenetic Proteins | ALL | B |
| | Fibroblast Growth Factors | B, K, M, P, T | B |
| | IL6/LIF | | B |
| | Insulin–like Growth Factors | ALL | B |
| | TGFs | B, K, M, P | B |
| | Tob | B, M, P | B |
| | Vascular Endothelial Growth Factor | B, M | X |
| Endocrine regulation of osteoblast function | Activin | B, L, M, P | B |
| | Estrogen receptor | ALL | |
| | Retinoic receptor X | B, P | B |
| | Steroidogenic factor | B, L, P, T | |
| | nuclear receptors (steroid/thyroid family) | | B |
| Transcription factor that regulates collagen type 1 synthesis | Y-box binding protein | B, K, M, P | B |

Multiorgan gene expression profiling in salmon calcitonin treated animals. Organs where changes in expression were seen are displayed. B= bone; K= kidney; M= muscle; P= pituitary; L= liver; T= trachea.

[00190] Three families of growth factors, the transforming growth factor betas (TGF-βs), insulin-like growth factors (IGFs), and bone morphogenetic proteins (BMPs), are considered to be principal local regulators of osteogenesis. Bone morphogenetic proteins are thought to have their major effects on early precursor bone cell replication and osteoblast commitment. In contrast, TGB-βs are thought to be the most potent inducers of committed bone cell replication and osteoblast matrix production, while IGFs appear to integrate and extend the effect of both factors. McCarthy TL *et al., Crit. Rev. Oral Biol. Med.* 11(4): 409-22 (2000). These results support the fact that both salmon calcitonin and PTS893 are able to regulate these local and systemic factors implicated in bone metabolism. The fact that salmon

calcitonin regulates α2-HS glycoprotein (AHSG), which blocks TGF-β-dependent signalling in osteoblastic cells, also supports this role. Mice lacking AHSG display growth plate defects, increased bone formation with age, and enhanced cytokine-dependent osteogenesis. Szweras M *et al., J. Biol. Chem.*, 277(22): 19991-19997 (2002).

[00191] Salmon calcitonin and PTS893 were also shown to modulate the expression of the genes coding for vascular endothelial growth factor (VEGF). VEGF is known for playing a key role in normal and pathological angiogenesis. The critical role of angiogenesis for successful osteogenesis during the endochondral ossification is well documented. VEGF indirectly induces proliferation and differentiation of osteoblasts by stimulating endothelial cells to produce osteoanabolic growth factors. Wang DS *et al., Endocrinology* 138(7): 2953-62 (1997). In addition, VEGF stimulates chemotactic migration of primary human osteoblasts, suggesting a functional role in bone formation and remodeling. Mayr-Wohlfahrt U *et al., Bone* 30 (3): 472-7 (2002).

[00192] The effects of parathyroid hormone on osteoblast for mediating both bone resorption and formation have been widely described. Swarthout JT *et al., Gene* 282(1-2):1-17 (2002). It was here possible to confirm the effect of PTS893 on cytokines like interleukin 6 (IL-6), which mediates the paracrine activation of osteoclast differentiation and activity. Greenfield EM *et al., Life Sci.* 65:1087-102 (1999). PTS893 also produced a strong up-regulation on nuclear receptors (steroid/thyroid family).

[00193] Both calcitonin and parathyroid hormone receptors belong to the G-protein receptor superfamily. After receptor stimulation, signal transduction is mediated by adenylate cyclase/cAMP/protein kinase, phospholipase C, phospholipase D, and MAPK (as a late effecter) pathways in the case of calcitonin, and by adenylate cyclase and phospholipase C in the case of parathyroid hormone. Gene profiling analysis allowed the reconstruction of these pathways, showing genes that were modulated by the treatment and that are localised at different levels of the signal transduction pathway.

TABLE 41
Effects on Signal Transduction and Cell Cycle

| Function | Coding Gene | Salmon Calcitonin | PTS893 |
|---|---|---|---|
| Signal transduction. | Adenylate cyclase | | B |
| | Calcyclin binding protein | | B |
| | Calreticulin | B, K, M | |
| | CREM | B, L, P | B |
| | CDC Kinase | B, M | |
| | MAPK | ALL | B |
| | Protein kinases | ALL | |
| | Phosphatidylinositol pathway | ALL | B |
| | Phosphodiesterase (IB, 4A, 4B) | ALL | B |
| | Phospholipase (C, D) | ALL | B |
| | PCNA | B | |
| | SMAD pathway | ALL | B |
| | STAT pathway | ALL | B |
| Cell cycle | Cyclins (A, A2, B1, C, D2, E2, G1, G2) | B | B |
| | Cyclin-dependent kinases 5, 6, 10 | B, K, P, T | B |
| | Cyclin-dependent kinases inhibitor 1A, 1C, 2D) | B | B |

Multiorgan gene expression profiling in salmon calcitonin treated animals. Organs where changes in expression were seen are displayed. B= bone; K= kidney; M= muscle; P= pituitary; L= liver; T= trachea.

[00194] Bone morphogenetic protein (BMP) controls osteoblast proliferation and differentiation through Smad proteins. Tob, a member of the emerging family of antiproliferative proteins, is a negative regulator of BMP/Smad signalling in osteoblasts. Smad pathway as well as Tob as one of their regulators were also identified as genes modulated by the sCT and PTS893 treatment, in agreement with the hypothesised effect of both compounds on BMP regulation of bone remodelling. Within this context, both compounds seem to exert a direct influence on cell cycle, since changes in cyclins and cyclin-related proteins could be also observed.

[00195] Both compounds regulate also synthesis and degradation of extracellular matrix components (TABLE 42).

TABLE 42
Effects on Extracellular Matrix

| Function | Coding Gene | Salmon Calcitonin | PTS893 |
|---|---|---|---|
| Cell attachment. Signal transduction. | Integrins | B, M, P | B |
| Collagen digestion | Collagenase | B | |
| | Matrix metalloproteinases I, II | B, L, P, T | |
| Collagen synthesis | Procollagen endopeptidase/proteinase | | B |
| | Lysyl hydroxylase | | B |
| Extracellular matrix component | Aggrecan | | B |
| | Cartilage Oligomeric Matrix Protein Precursor | B, K, | |
| | Collagen type I, type II, type III, type IV, type V, type VI, type IX, type X, type XI, type XIII, type XIV, type XV, and/or type XVI) | ALL | B |
| | Chondroitin sulphate proteoglycan | K, M, T | B |
| | Dermatopontin | | B |
| | Heparan sulphate proteoglycan | L,T | B |
| | Syndecan | | B |

Multiorgan gene expression profiling in salmon calcitonin-treated animals. Organs where changes in expression were seen are displayed. B= bone; K= kidney; M= muscle; P= pituitary; L= liver; T= trachea.

[00196] Of particular interest is the regulation of the Y-Box binding protein (YB-1), which appears to be modulated by both treatments and in four out of six organs analysed in the salmon calcitonin group. YB-1 is a protein that interacts with a TGF-β response element in the distal region of the collagen alpha 1(I) gene. YB-1 protein activates the collagen promoter and translocates into the nucleus during TGF-β addition to fibroblasts, suggesting a role for this protein in TGF- β signalling. Sun W *et al., Matrix Biol.* 20(8): 527-41 (2001).

[00197] In addition, salmon calcitonin and PTS893 regulated some aspects of the mineralization of the bone extracellular matrix, since changes in amelogenin, dentin and ectonucleotide pyrophosphatases were observed.

TABLE 43
Effects on Mineralization and Visualisation

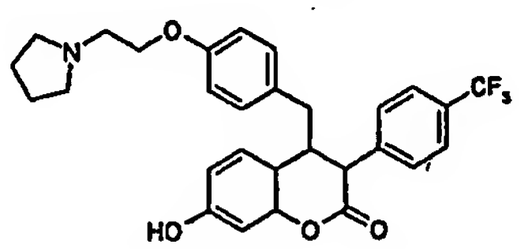
| Function | Coding Gene | Salmon Calcitonin | PTS893 |
|---|---|---|---|
| Cement component | Amelogenin | B, L | B |
| Mineral matrix protein | Dentin | B | B |
| Enzyme for synthesis of inorganic Pi | Ectonucleotide pyrophosphatases | B, M | |
| Growth factor vascularization | VEGF | B, M | B |

Multiorgan gene expression profiling in salmon calcitonin treated animals. Organs where changes in expression were seen are displayed. B= bone; K= kidney; M= muscle; P= pituitary; L= liver; T= trachea.

## EXAMPLE VI

## GENE PROFILING OF AFI030

[00198] AFI030 has the chemical formula 7-hydroxy-4-[4-(2-pyrrolidin-1-yl-ethoxy)-benzyl]-3-(2,4-dichloro-phenyl)-chromen-2-one, as shown:




[00199]

[00200] AFI030 was developed for the treatment of bone-resorbing diseases generally, including osteoporosis, metastatic bone cancer, osteolytic lesions with orthopedic implants, Paget's disease, and bone loss associated with hyperthyroidism. AFI030 can also be used to treat other conditions associated with IL-6 including various cancers (*e.g.* breast cancer, prostate cancer, colon cancer, endometrial cancer, multiple myeloma, renal cell carcinoma, and cervical carcinoma) and arthritis (*e.g.* adjuvant-, collagen- and antigen-induced arthritis, particularly rheumatoid arthritis). See, published PCT patent applications WO 96/31206, WO 00/39120, WO 01/49673. AFI030 has estrogenic, antiestrogenic, antifertility and uterotropic activity.

[00201] The purpose of this EXAMPLE is to identify the "compound signature" of AFI030, to define a strategy for further development. The main concerns are the potential stimulation of the uterus and the induction of deep venous thrombosis (DVT). In addition, a goal of the EXAMPLE is to identify biomarkers for efficacy or risk assessment of AFI030.

[00202] Selective Estrogen Receptor Modulators (SERMs) are selective estrogen receptor modulators that act as agonists or antagonists depending on the target tissue. To increase our understanding of the direct tissue-specific effects of different SERMs and to elucidate the underlying molecular mechanisms, a study was started to perform a comprehensive in vivo gene expression profiling using DNA microarrays. Ovariectomized (OVX) rats (n=4) were treated p.o. with 0.3 mg/kg α-ethinyl estradiol (αEE), 3 mg/kg raloxifene, 0.1 mg/kg NVP-AFI030, 1 mg/kg tamoxifen or vehicle.

[00203] *Methods*. A 4-week gene profiling test was performed on in ovariectomized (OVX) female cynomolgus monkeys. The mode of administration: p.o. (gavage). The compounds administered were estradiol (αEE) at 0.4 mg/kg/day; tamoxifen at 10 mg/kg/day;

raloxifene at 10 mg/ kg/day; and AFI030 in two groups, at 1 mg/ kg/day (AFI030 LD) and at 10 mg/ kg/day (AFI030 HD). A sham control was also performed. Four animals were used for each group. The results were compared to a control ovariectomized monkeys.

[00204] After 4, 8 and 24 hours, animals were sacrificed and nineteen tissues were dissected including adrenal gland, bladder, blood, bone marrow, brain cortex, cortical bone (femur), duodenum, heart, hippocampus, hypothalamus, ileum, kidney, liver, muscle (hind limb), skin (neck), pancreas, pituitary, uterus and vagina. In addition, tissues were collected from sham controls. A total of 120 organs were collected and frozen,

[00205] Clinical investigations (hematology, clinical chemistry and hormone determinations) were performed on days -6, 1 and 28.

[00206] The results are shown in FIGS. 3 and 4. The statistics for FIG. 3 are shown in TABLE 44.

TABLE 44

Statistics for FIG. 3; Increase in Endometrial Thickness Following the Administration of Estrogenic Compounds to Ovariectomized monkeys

| Dunnett's Multiple Comparison Test | P value |
|---|---|
| Raloxifene vs sham | P > 0.05 |
| Raloxifene vs ovariectomized monkeys | P < 0.05 |
| Raloxifene vs estradiol | P < 0.01 |
| Raloxifene vs tamoxifen | P < 0.01 |
| Raloxifene vs AFI030 LD | P < 0.01 |
| Raloxifene vs AFI030 HD | P > 0.05 |

[00207] The results show that estradiol, tamoxifen and AFI030 LD increase endometrium thickness. Raloxifene and AFI030 HD are less stimulatory. The increase in endometrial thickness is significant as compared with ovariectomized monkeys.

[00208] The statistics for FIG. 4 are shown in TABLE 45.

TABLE 45

Statistics for FIG. 4; Increase in Endometrial Epithelial Height Following the Administration of Estrogenic Compounds to Ovariectomized monkeys

| Dunnett's Multiple Comparison Test | P value |
|---|---|
| Raloxifene vs sham | P > 0.05 |
| Raloxifene vs ovariectomized monkeys | P > 0.05 |
| Raloxifene vs estradiol | P < 0.01 |
| Raloxifene vs tamoxifen | P < 0.05 |
| Raloxifene vs AFI030 LD | P < 0.01 |
| Raloxifene vs AFI030 HD | P > 0.05 |

[00209] The results show that estradiol, tamoxifen and AFI030 LD increase epithelial height. AFI030 HD also increases epithelial height, but this increase is not significant. These results are consistent with previous results from similar profiling in an eight week test in ovariectomized rats. See, FIGS 5 and 6. The endometrial effects not differentiated in ovariectomized rats.

[00210] The results of the administration of compounds showed the modulation of gene expression. The impact of ovariectomy and the resulting deprivation of estrogens was a slight to moderate down-regulation of estrogen-related genes, including components of the extracellular matrix, growth factors and signal transduction pathways, such as integrin signaling and the secretion of Frizzled-related proteins.

[00211] Among the genes tested for gene expression were extracellular components, such as nidogen (enactin); glypican 1; fibrillin 1; lysyl oxidase (for the metabolism of collagen); chondroitin sulfate proteoglycan 2 (versican); extracellular matrix protein 1; SPARC; dermatopontin; fibronectin 1; elastin; spondin 2; bone morphogenetic protein 1; and collagens I, III, IV, V, VI, VIII, XV and XVIII. Also among the genes tested for gene expre4ssion were growth factors, such as cysteine-rich, angiogenic inducer, 61 (CYR61); epidermal growth factor receptor pathway substrate 8-related protein 1; epithelial membrane protein 1; granulin; immediate early response 3; latent transforming growth factor beta binding protein 1; tumor necrosis factor receptor superfamily, member 12A; insulin-like growth factor binding protein 2, 4 and 5; and insulin-like growth factor 1 and 2. Among the estrogen-regulated signaling pathways tested were those for progesterone receptors; nuclear receptor co-activator; annexins A1, A2, A3, A5, A6 and A8; the Wnt/Frizzled pathway, including secreted Frizzled proteins, DSH, LRP 4, 8 and 10; protocadherin 9 and 16, and catenin; and integrin signaling pathways.

[00212] Estradiol reverts the effect of ovariectomy, with a marked up-regulation of the genes down-regulated in the ovariectomized monkeys and the induction of other genes. (The induction may be due to the dose of estradiol used, 0.4 mg/kg/day). The induction of estrogenic effects on gene expression was stronger with AFI030 at 1 mg/kg/day and tamoxifen at 10 mg/kg/day. The induction of estrogenic effects on gene expression was weaker with AFI030 or raloxifene at 10 mg/kg/day.

[00213] The results of an initial gene profiling are shown in TABLE 46, which list the data as fold increase above ovariectomized monkeys.

TABLE 46
Gene Profiling Study in Monkeys – Uterus Data

| | Sham Control | Ethinyl estradiol | AFI030 LD | Tamoxifen | AFI030 HD | Raloxifene |
|---|---|---|---|---|---|---|
| collagen, type I, alpha 1 | 2.47 | 6.55 | 4.12 | 3.42 | 1.75 | 1.22 |
| collagen, type I, alpha 2 | 2.76 | 5.44 | 4.80 | 3.81 | 2.88 | 1.90 |
| insulin-like growth factor 1 | 1.83 | 6.01 | 4.78 | 4.21 | 2.98 | 2.17 |
| progesterone receptor | 2.62 | 10.39 | 6.54 | 4.52 | 4.02 | 2.02 |
| Estrogenic effect: higher to lower | | 1 | 2 | 3 | 4 | 5 |

[00214] The most estrogenic compounds and dosages were estradiol (αEE); tamoxifen; and AFI030 LD. A somewhat estrogenic compound and dosage is AFI030 HD. The least estrogenic compound and is raloxifene.

[00215] Thus, the results of the gene expression profiling experiment and of the histology study are in line. They both indicate that tamoxifen and AFI030 LD (1 mg/kg) cause the strongest estrogenic effects in the uterus. AFI030 HD induces smaller estrogenic effects and raloxifene is the least estrogenic compound in the uterus.

[00216] Finally, the administration of estradiol reduces serum C-telopeptide and alkaline phosphatase by up to 40%. See, FIGS 7 and 8. Raloxifene and AFI030 HD have similar efficacy and are not as effective as estradiol.

[00217] *Summary*. The results of this EXAMPLE show that (1) In the uterus, several genes are down-regulated by ovariectomy and up-regulated by estradiol. (2) The strongest estrogenic effects are seen with AFI030 at 1 mg/kg/day (AFI030 LD) and tamoxifen. (3) The induction of estrogenic effects is smaller with AFI030 at 10 mg/kg/day (AFI030 HD). (4) Raloxifene shows the weakest estrogenic effect in uterine tissue.

[00218] All references cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes. In addition, all GenBank accession numbers, Unigene Cluster numbers and protein accession numbers cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each such number was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

[00219] The present invention is not to be limited in terms of the particular embodiments described in this application, which are intended as single illustrations of individual aspects of the invention. Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. Functionally equivalent methods and apparatus within the scope of the invention, in addition to those enumerated herein, will be apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications and variations are intended to fall within the scope of the appended claims. The present invention is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.

## CLAIMS

We claim:

1. A business method for identifying a compound as a candidate for pharmaceutical development, comprising the steps of:
   (a) administering a compound to one or more test animals;
   (b) obtaining the gene expression patterns induced by administration of the compound in organs from the test animals; and
   (c) identifying the function of the compound *in vivo,* wherein the identification of the function of the compound *in vivo* indicates whether the compound is a candidate for pharmaceutical development.

2. The method of claim 1, wherein the step of obtaining the gene expression pattern further comprises:
   comparing the gene expression patterns of the test animals to a control gene expression pattern.

3. The method of claim 1, wherein the identity of the compound is not known by the administrator.

4. The method of claim 1, wherein the function of the compound is not known by the administrator.

5. The method of claim 1, wherein the compound is a protein or a peptide.

6. The method of claim 1, wherein administration is the direct administration of the compound.

7. The method of claim 1, wherein administration is the indirect administration of the compound.

8. The method of claim 1, wherein the test animals are selected from the group consisting of mouse, rat and monkey.

9. The method of claim 1, wherein the test animals comprise either both mice and monkeys or both rats and monkeys.

10. The method of claim 1, wherein the obtaining of the gene expression pattern is by organism-wide gene expression profiling.

11. The method of claim 1, wherein the test animals are mice and wherein the gene expression patterns of at least twenty-five organs are obtained.

12. The method of claim 1, wherein the test animals are monkeys and wherein the gene expression patterns of at least 120 organs are obtained.

13. The method of claim 1, wherein the comparing of the gene expression patterns of the test animals comprises an analysis of multiple targets and indications.

14. The method of claim 1, wherein the comparing of the gene expression patterns of the test animals comprises integrating information from genomic databases.

15. The method of claim 1, wherein the identifying of the function of the compound comprises an initial step of excluding from further analysis those genes whose values are systematically in the lower expression ranges where the experimental noise is high

16. The method of claim 1, wherein the identifying of the function of the compound comprises the step of selecting a threshold t-test p-value that identifies genes with different values between treated and non-treated based on a two component error model.

17. A system for identifying a compound as a candidate for pharmaceutical development, comprising:

(a) an apparatus for obtaining the gene expression patterns induced by administration of the compound in organs obtained from the test animals, the gene expression patterns being stored in digital format in a memory storage device;

(b) a database comprising stored the gene expression patterns of animals (i) having the same or different predetermined genetic composition from said test animals and (ii) which have been exposed to either control conditions and/or treatment. with compounds similar to the test compounds;

(c) means for comparing the collected gene expression patterns with the stored gene expression patterns and determining the presence or absence of correlations; and

(d) means for determining the function of the test compound on the basis of the presence or absence of the correlations, wherein the identification of the function of the compound *in vivo* indicates whether the compound is a candidate for pharmaceutical development.

## ABSTRACT

A discovery process beginning with an *in vivo* screening of proteins, peptides, natural products, classical medicinal compound or other substances. The administration of compounds to the animal can be either direct or indirect, such as by the administration and expression of cDNA-containing plasmids. Since the discovery process of the invention is based on a non-preconceived hypothesis and whole organism multi-organ analysis, a compound can be selected for testing in the absence of any biological selection criteria. The resulting organism-wide pattern of the gene expression changes in the transcriptome provides an overview of the activities at the molecular and organism-wide levels. The discovery process of the invention then integrates *in vivo* profiling and internal and external genomic databases to elucidate the function of unknown proteins, typically within few months.

**LTBP2: latent transforming growth factor beta binding protein 2**

**human vs mouse**

```
GPA018   1 qrdpvgryepaggdanrlrrpggsypaaaaakvyslfreqdapvaglqpv 50
           ||| :|||||| ||||| | ||:|||||||||||||| |||| || |
mLTBP2  56 qrdsigryepasrdanrlwhpvgshpaaaaakvyslfrepdapvpglsps 105

           51 eraqpgwgsprrpteaearrpsraqqsrr 79
              |  ||  |.|    |||||||| | || ||
          106 ewnqpaqgnpgwlaeaearrpprtqqlrr 134
```

**hypothetical protein XP_097406  No match in mouse**

```
GPA019   1 atlggpeeestienyasrpe.............afkadeflnwhalfes 36
           ||||||||||||||||||||             ||||||||||||||||
XP_097406 23 atlggpeeestienyasrpeafntpflnidklrsafkadeflnwhalfes 72

           37 ikrklpflnwdafpklkglrsatpdaq 63
              ||||||||||||||||||||||||||||
           73 ikrklpflnwdafpklkglrsatpdaq 99

GPA020   1 atlggpeeestienyasrpeafntpflnidklr.afkadeflnmhalfes 49
           ||||||||||||||||||||||||||||||||| ||||||||| ||||||
XP_097406 23 atlggpeeestienyasrpeafntpflnidklrsafkadeflnwhalfes 72

           50 ikrklpflnwdafpklkglrsatpdaq 76
              ||||||||||||||||||||||||||||
           73 ikrklpflnwdafpklkglrsatpdaq 99
```

**ANGPTL1: angiopoietin-like 1**

**human vs mouse**

```
GPA022   1 CAYTFLVPEQRITGPICVNTKGQDASTIKDMITRMDLENLKDVLSRQKR. 49
                                                ||||||||||||||||||
mANGPTL1 1 CSYTFLVPEQRITGPICVNTKGQD AGTIKD.mitrmdlenlkdvlsrqkre 20

GPA023   51 stikdmitrmdlenlkdvlsrqkr..................... 74
              ||||||||||||||||||||||
mANGPTL1 1 ....mitrmdlenlkdvlsrqkreidvlqlvvdvdgnivnevkllrkes 45
```

Translated mouse 5' sequence

Human precursor sequence

**FIG. 1**

# GPA018 IN KIDNEY: DIFFERENTIALLY EXPRESSED GENES WITH RELATION TO TGFβ SIGNALING

| Description | p-value | FC | Treatment: GPA018 | Treatment: control | Probe set ID |
|---|---|---|---|---|---|
| vitamin D receptor | 0.019 | 2.30 | 226.194 (128.4 to 298.52) | 120.65 (32.02 to 278.91) | 99965_at |
| bone morphogenic protein receptor, type II | 0.036 | 2.00 | 87.798 (42.42 to 168.21) | 47.956 (14.39 to 106.76) | 99865_at |
| cadherin 5 | 0.005 | -1.32 | 78.321 (59.21 to 109.76) | 102.412 (86.02 to 120.66) | 104083_at |
| lymphoid enhancer binding factor 1 | 0.010 | 1.56 | 145.94 (108.53 to 171.06) | 97.804 (57.08 to 155.06) | 103628_at |
| catenin src | 0.049 | 1.30 | 124.611 (109.53 to 147.6) | 98.962 (48.99 to 128.24) | 98151_s_at |
| calcium/calmodulin-dependent serine protein kinase | 0.049 | -1.31 | 88.542 (50.23 to 119.39) | 113.935 (90.22 to 167.09) | 102248_f_at |
| MAP kinase-interacting serine/threonine kinase 2 | 0.048 | 1.29 | 205.875 (181.61 to 249.07) | 164.699 (99.8 to 208.85) | 92473_at |
| mitogen-activated protein kinase 12 | 0.039 | -1.19 | 72.744 (52.6 to 87.22) | 86.312 (69.76 to 108.47) | 92323_at |
| mesoderm development candiate 2 | 0.041 | 1.22 | 238.53 (178.42 to 283.5) | 196.015 (143.39 to 269.01) | 95405_at |
| forkhead box D2 | 0.037 | 1.20 | 118.66 (96.56 to 152.03) | 98.895 (75.88 to 129.5) | 94621_at |
| Fas death domain-associated protein | 0.050 | -1.21 | 77.96 (56.46 to 100.72) | 94.311 (73.12 to 115.51) | 96125_at |
| ephrin B2 | 0.021 | -1.28 | 75.761 (54.43 to 102.77) | 95.971 (69.63 to 113.05) | 160857_at |
| tyrosine kinase receptor 1 | 0.009 | -1.32 | 74.769 (58.19 to 98.31) | 99.618 (74.5 to 131.81) | 161184_f_at |
| biglycan | 0.015 | -1.36 | 152.224 (112.82 to 207.61) | 208.264 (155.91 to 280.86) | 162346_f_at |
| Tnf receptor-associated factor 5 | 0.008 | -1.39 | 93.96 (68.53 to 144.09) | 129.381 (94.61 to 154.04) | 103255_at |
|  | 0.005 | 1.13 | 5345.84 (4783.18 to 6115) | 4793.84 (4472.95 to 4995.46) | 93063_at |
| actin, beta, cytoplasmic | 0.002 | 1.57 | 861.716 (470.94 to 1'102.93) | 539.534 (368 to 641.11) | 95705_s_at |
| integrin beta 5 | 0.023 | -1.14 | 192.941 (167.13 to 215.24) | 219.201 (191.49 to 252.53) | 161786_f_at |
| integrin beta 5 | 0.048 | -1.20 | 348.915 (309.8 to 427.48) | 422.901 (311.02 to 527.04) | 100601_at |
| procollagen, type IV, alpha 4 | 0.045 | 1.42 | 149.262 (124.51 to 175.51) | 111.732 (54.42 to 160.81) | 101774_at |
| spectrin beta 2 | 0.004 | 1.12 | 829.471 (759.59 to 928.53) | 741.779 (642.34 to 791.98) | 93571_at |
| talin | 0.032 | 1.12 | 542.186 (490.64 to 593.16) | 486.978 (420.91 to 561.39) | 99448_at |
| myosin VIIa | 0.042 | -1.13 | 132.021 (116 to 166.57) | 148.674 (126.49 to 166.78) | 94713_at |
| transgelin | 0.045 | -1.26 | 158.629 (138.26 to 177.61) | 205.163 (132.68 to 308.45) | 93541_at |

FIG. 2

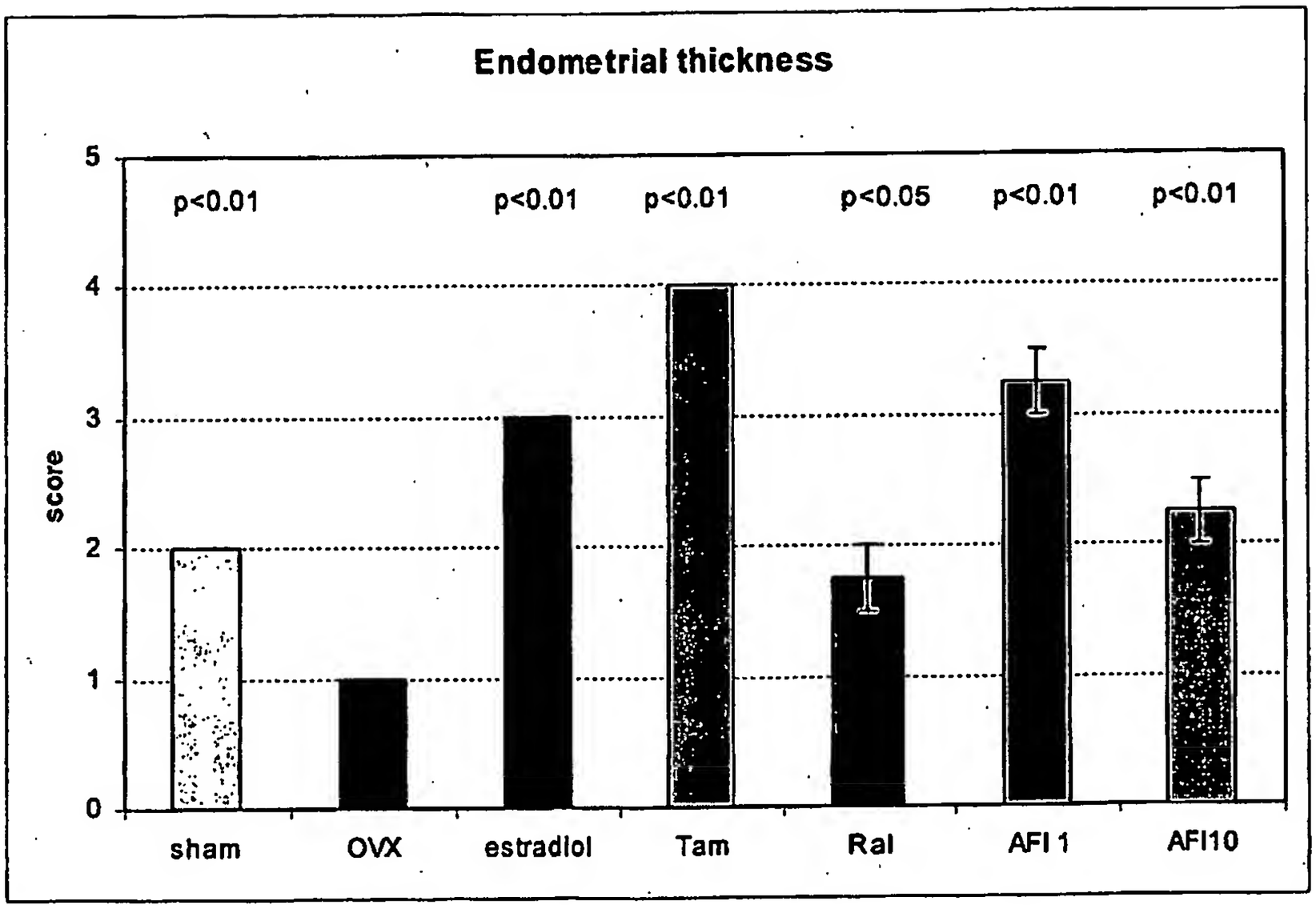



FIG. 3

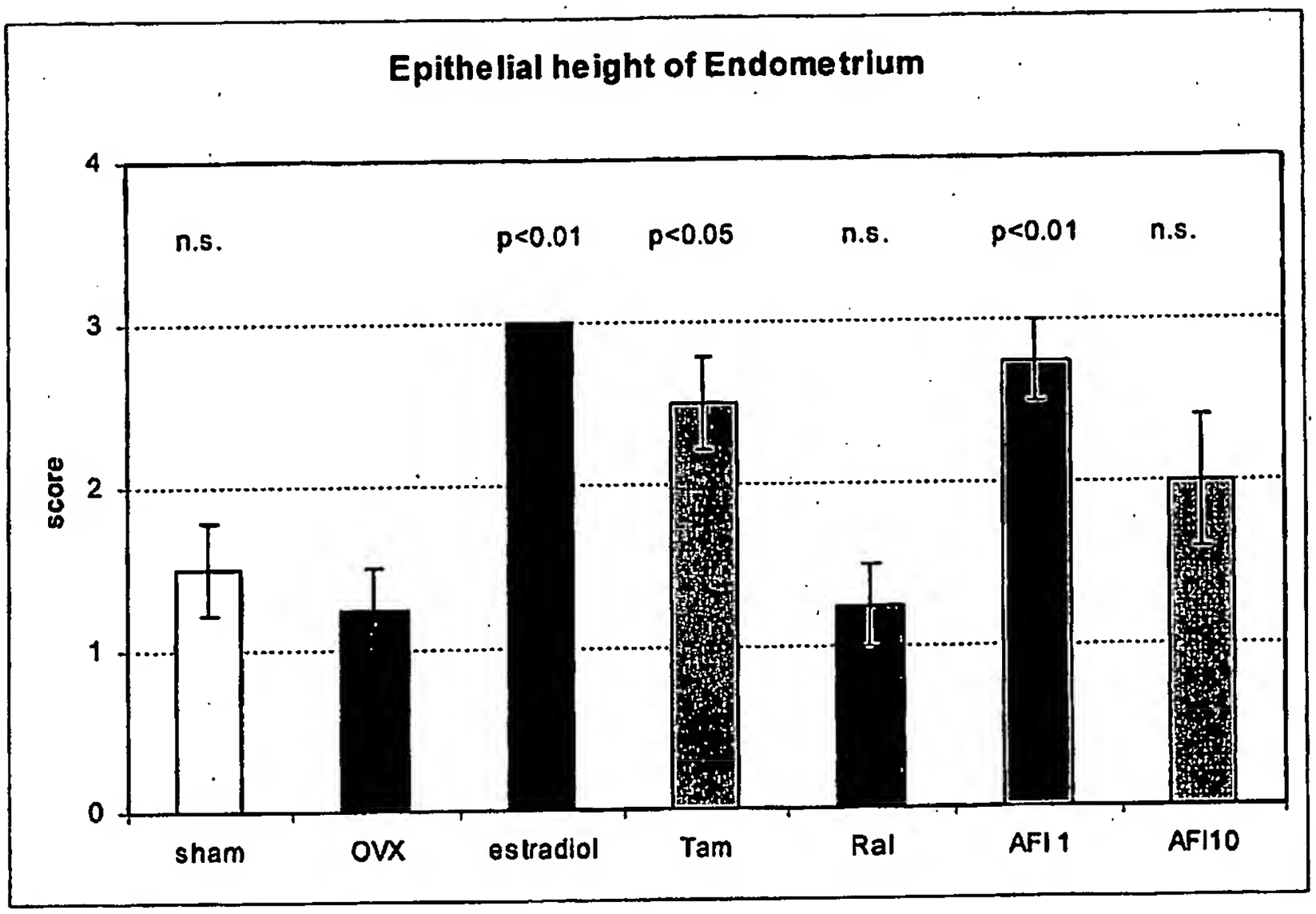



FIG. 4

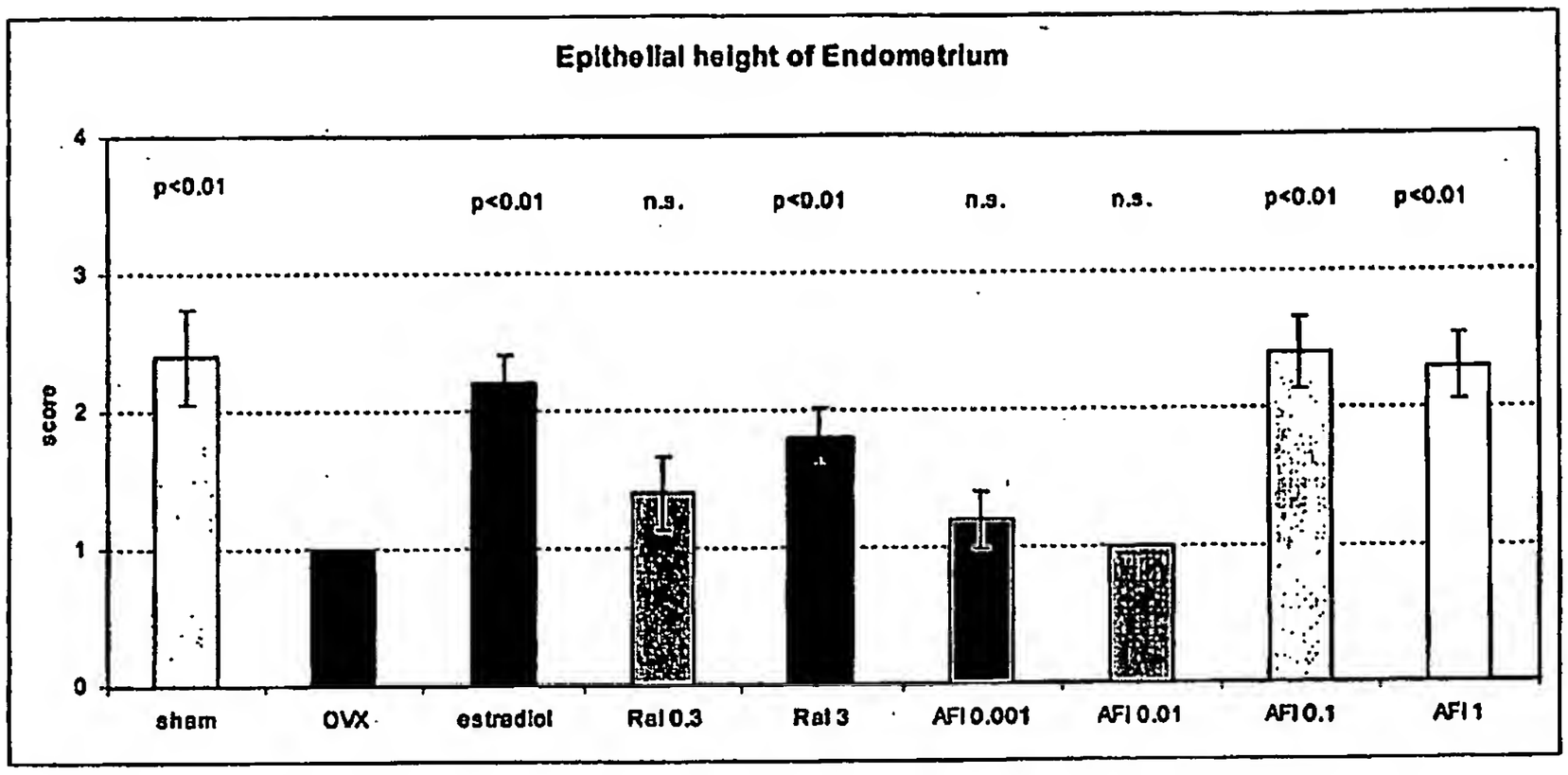



FIG. 5

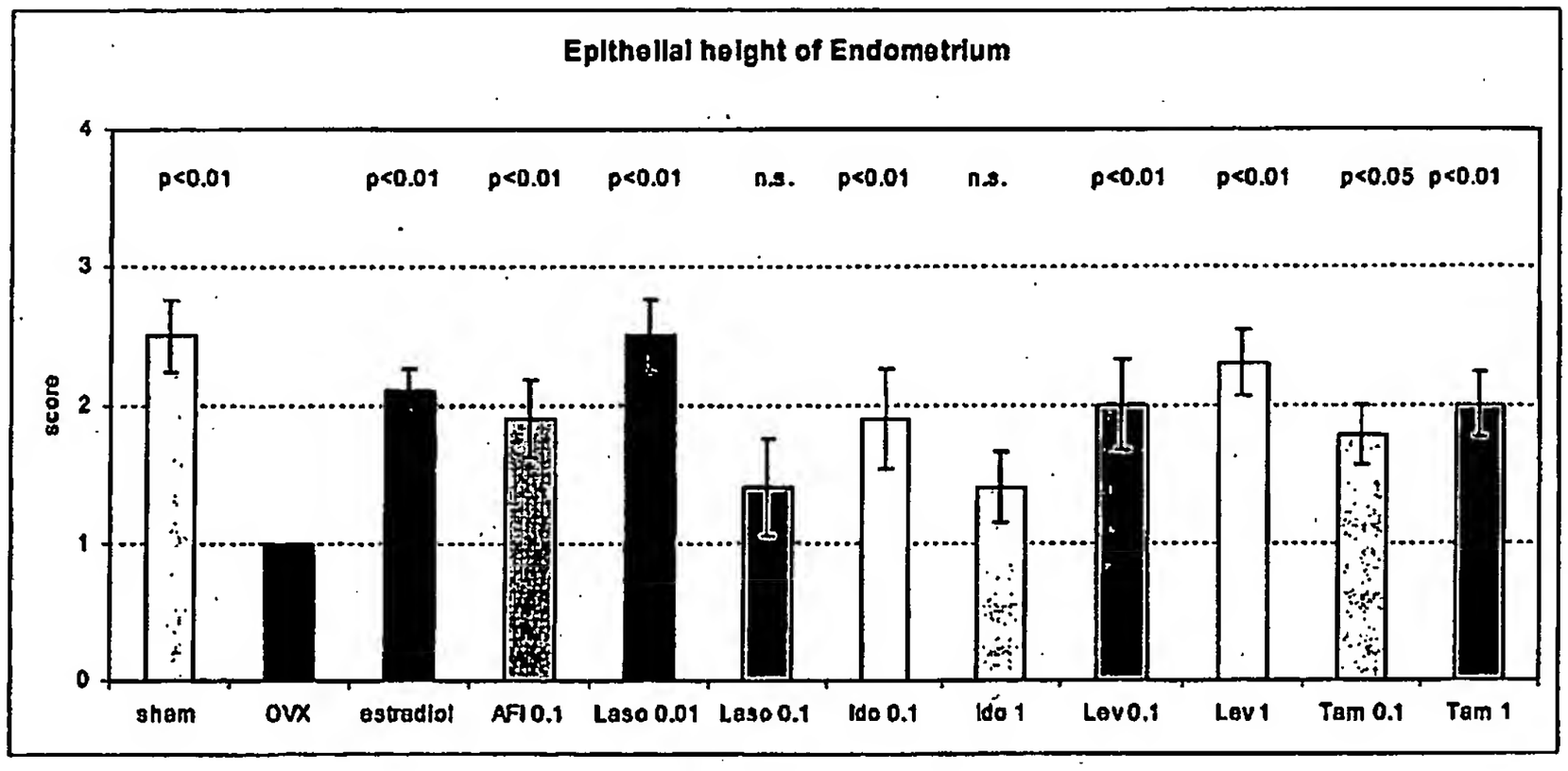



FIG. 6

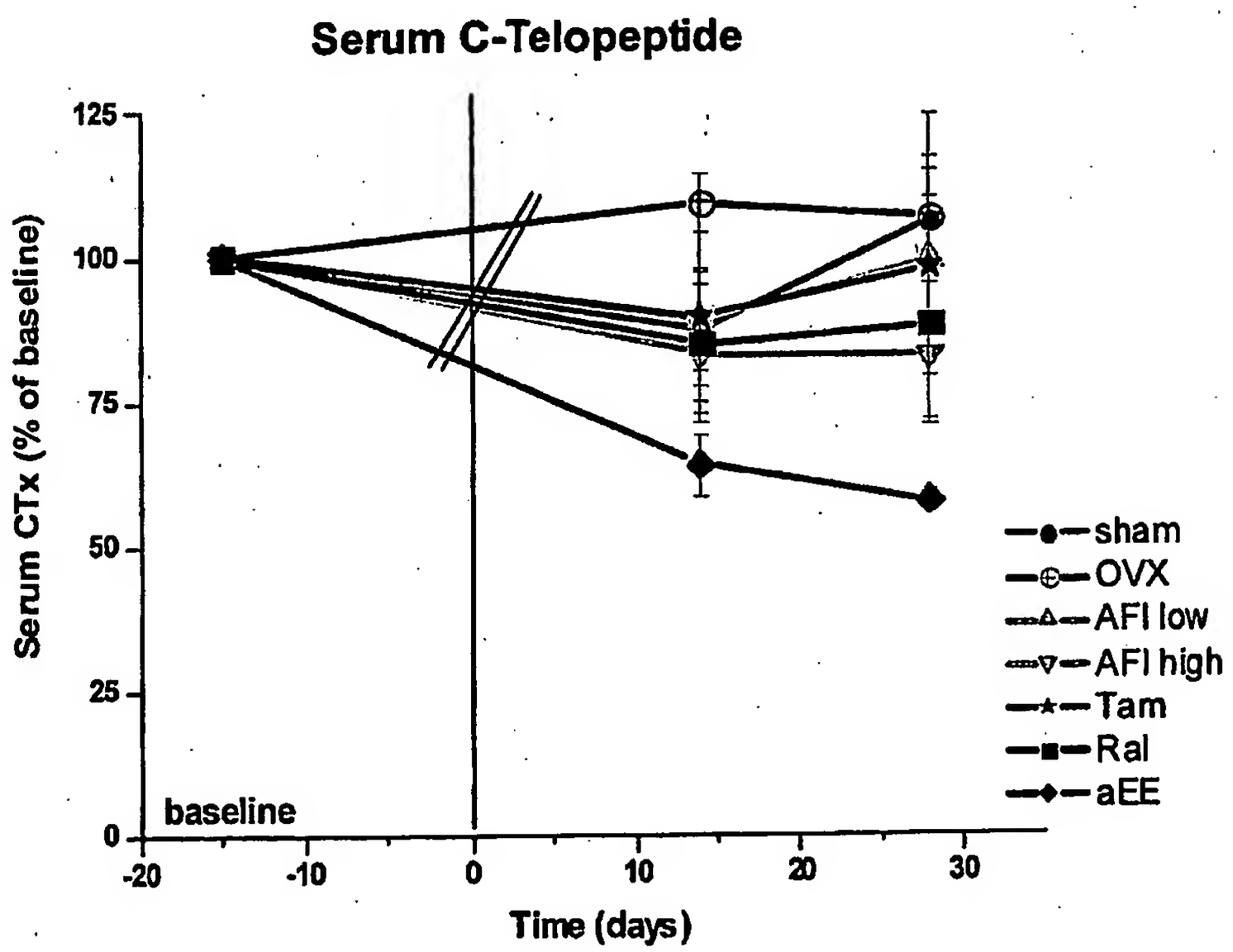



FIG. 7

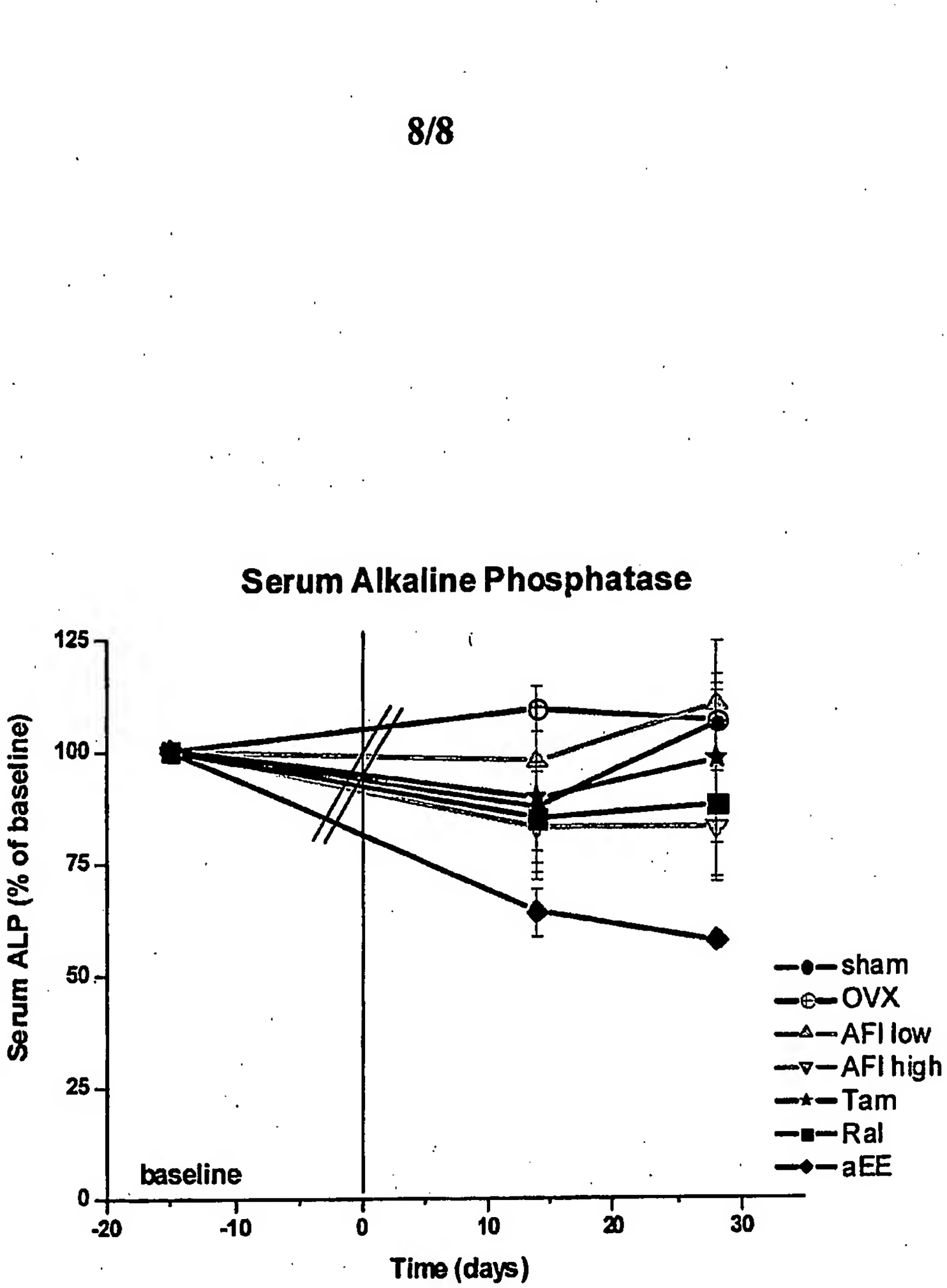



FIG. 8